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ON THE RELATION BETWEEN TONUS AND SMOOTH MUSCLE IN THE TERRAPIN HEART

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The effects of definite changes in the hydrogen-ion concentration of the perfusate to isolated terrapin hearts were reported by one of us in a previous paper (1). The results while corroborating those of others were new in so far as they set more precise limits to the P_H index of the perfusate than hitherto had been done. The effects on the heart concerned chiefly tonus. In the report the literature on tonus waves in the chelonian heart was briefly reviewed. The question as to the *locus operandi* of these waves in the heart wall was discussed but no final opinion could be given at that time.

In view of the fact however that changes in H-ion concentration are known to have much more marked effects upon smooth than upon cross-striped muscle it was thought that the tonus effects observed may be due for the most part to a specific action of the H-ion concentration upon smooth rather than upon the cross-striped muscle in the heart wall. The tonus changes it will be remembered are most marked in the auricular walls where the smooth fibers are most abundant (2). The ventricular walls also show tonus response but to a much less extent, and it is just here where most probably some smooth muscle also is present but more sparsely distributed.

If this interpretation of the phenomena in question were the correct one then it ought to follow that if a poison could be applied to the heart that would specifically paralyze the smooth muscle, leaving the cross striped fibers practically unaffected, a perfusate of the appropriate P_H value no longer would elicit tonus

waves. Among the opium alkaloids and some of the benzyl compounds we have such drugs.

According to Pal (3), and more recently Macht (4), the opium alkaloids are sharply divisible into two groups regarding their action on smooth muscle. One, the pyridin-phenanthrene group, of which morphine is the more important, stimulates the contractile activity of smooth muscle organs whereas the second group, containing papaverin and benzyl-isoquinaline derivatives, depresses and inhibits the contractile activity of the same organs. On the other hand the action of these drugs upon cross-striped cardiac tissue appears to be just the reverse, that is, the morphine group depresses while the papaverin group in the lower concentrations stimulates.

In a word, if the high state of tonus produced by a chosen hydrogen-ion concentration of the perfusing fluid is due to the response of the cross striped tissue in the heart wall then the further addition of papaverin or benzyl alcohol to the perfusate ought not diminish the tonus in any way. If on the other hand the state of tonus is due to the response of the smooth muscle moiety in the heart wall then the addition of these drugs ought to lessen the tonus or even to cause it totally to disappear.

With the problem and its possible solution thus clearly in mind the experiments herein reported were undertaken.

During the progress of the experiments consideration of the character of the tonotropic nerve fibers supplying the heart walls could not be passed by and this too received some experimental tests.

EXPERIMENTAL

Terrapin hearts were perfused with Ringer's solution using the method described by Andrus (1). Proper care was taken to insure constant pressure conditions and to eliminate the effect of tonus changes in one chamber upon the record of another. The reaction of the perfusate in all cases was controlled by means of phenol-sulphone-phthalein. Whenever an alkaloid was employed to lower the tonus it was added to a Ringer solution of a P_H value which would in itself increase the tonus. Conversely whenever

an alkaloid was employed to raise the tonus it was added to an identical Ringer solution of a P_H value that in itself would lower tonus. It will be remembered that a solution of a 7.3 to 7.4 index (or lower) abolishes tonus, one of a 7.6 index promptly reestablishes it.

The effect of papaverin was first observed. A heart was first thrown into high tonus by perfusing with plain Ringer's, P_H index 7.6. Then the perfusing stream was changed to one quite identical except that papaverin hydrochlorid had been added to a

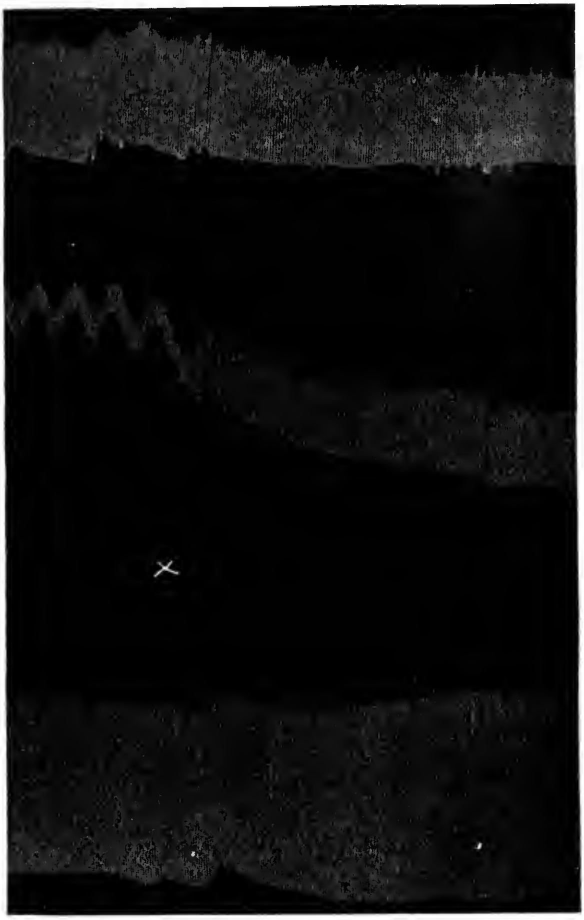
TABLE 1

Table showing fall in tonus following administration of papaverin 0.001 per cent in Ringer's, $P_H = 7.6$. Figures represent average of a number of measurements

PERFUSATE	VENTRICLE		LEFT AURICLE		RIGHT AURICLE	
	Tonus	Amplitude	Tonus	Amplitude	Tonus	Amplitude
I. $P_H = 7.4$	27	22	21	20	20	10
$P_H = 7.6$	27	19	27	12	25	8
Papaverin 0.001 per cent	27	17	14	9	22	5
II. $P_H = 7.4$	25	20	25	10	25	5
$P_H = 7.6$	35	15	35	5	25	5
Papaverin 0.001 per cent	25	15	5	3	19	3
$P_H = 7.6$	27	15	25	5	18	4
III. $P_H = 7.4$	7	20	20	5	20	10
$P_H = 7.6$	25	15	30	3	40	3
Papaverin 0.001 per cent	17	17	10	2	24	5
$P_H = 7.6$	18	18	20	4	24	5

concentration of 0.001 per cent. The results of a series of such experiments are put together in table 1 and a typical drum record is shown in figure 1. In every case the papaverin in the above concentration obliterated the tonus oscillations completely and augmented the amplitude of contractions. A change of the perfusate back to a papaverin-free Ringer ($P_H = 7.6$) caused a return of the tonus, as is shown in the table.

Next the antagonistic actions of morphine and papaverin were observed in the same manner. To a Ringer solution of a P_H index of 7.3 was added enough morphine sulphate to make a

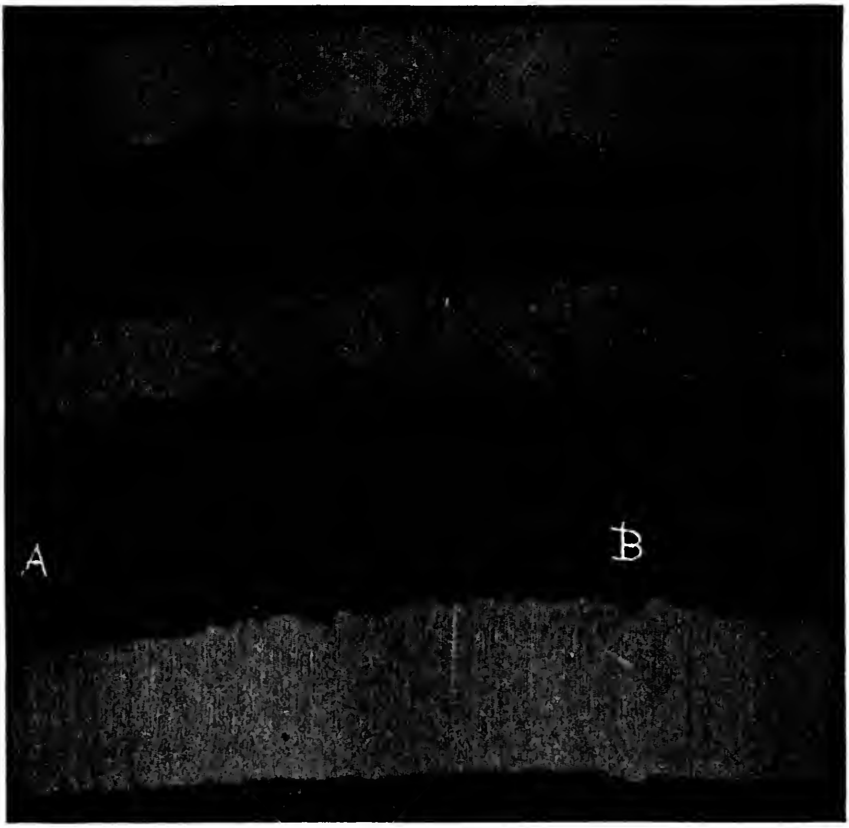


A

Fig. 1. Shows the Effect of Changing the Perfusate from a Ringer's Solution with a P_H Value of 7.6 to Another of the Same Composition Excepting the Addition of Papaverin Hydrochlorid to 0.001 Per Cent, at the Cross Mark

The upper tracing is of the right auricle, the middle one of the left auricle, and the lower one is of the ventricle. This order of tracings holds good for the other figures.

concentration of 0.01 per cent. This was passed through the heart until the tonus oscillations were more or less pronounced and this was followed by a papaverin containing perfusate made up as described above. Morphine invariably produced a rise of



A B

FIG. 2. SHOWS THE ANTAGONISTIC ACTION OF MORPHINE AND PAPAVERIN UPON TONUS

At *A* morphine sulphate, 0.01 per cent, was introduced in the perfusing stream, at *B* papaverin hydrochlorid, 0.001 per cent, was introduced.

tonus that was inhibited promptly upon changing to the papaverin solution, as is shown in figure 2. The experiment was repeated frequently and always the tonus could be raised or lowered

by simply alternating the perfusates. As others have observed for smooth muscle here too it was observed that papaverin was decidedly more effective than morphine so that a 0.001 per cent solution of the former counteracted the effect of a 0.01 per cent solution of the latter.

That the relaxing effect of the papaverin group on smooth muscle was due to the benzyl grouping in the molecule was pointed out by Macht (4) who obtained the same effects upon a variety of smooth muscle structures when benzyl esters were substituted for the papaverin. Benzyl alcohol was also shown to act in the same sense and since it is easily soluble in water it was

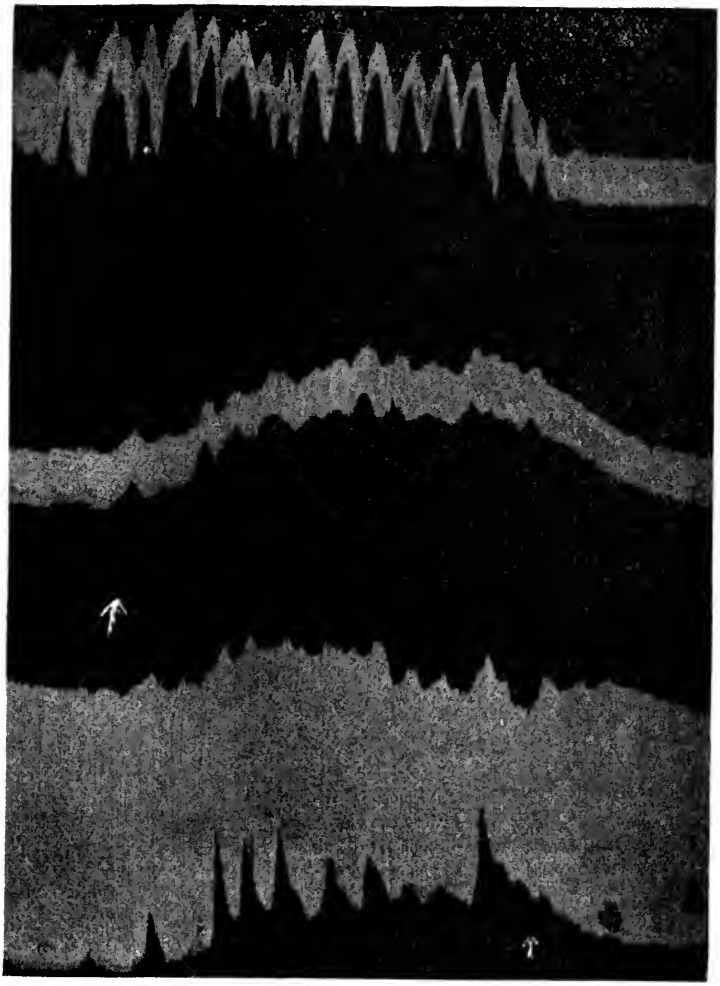
TABLE 2

Table showing effect of administration of benzyl alcohol following P_H 7.6 or morphine. Morphine = 0.01 per cent morphine sulphate in Ringer's, P_H = 7.3. Benzyl alcohol = 0.002 per cent in Ringer's, P_H = 7.6

PERFUSATE	VENTRICLE		LEFT AURICLE		RIGHT AURICLE	
	Tonus	Amplitude	Tonus	Amplitude	Tonus	Amplitude
IV. P_H = 7.4	12	36	10	7	16	8
P_H = 7.6	20	27	22	6	23	4
Benzyl alcohol, 0.002 per cent	14	32	12	7	10	7
V. P_H = 7.4	13	8	10	0	12	4
Morphine, 0.01 per cent	16	6	15	0	18	3
Benzyl alcohol, 0.002 per cent	13	9	11	0	10	4

used in our experiments. Its action was tested upon the increase of tonus elicited in the terrapin heart by a Ringer of a P_H index of 7.6, and in other cases by a Ringer containing morphine. The results are brought together in table 2; the drum records are illustrated by figures 3 and 4. In all cases benzyl alcohol had the same effect as papaverin, namely obliteration of tonus waves and augmentation of amplitude of beat.

The results of the foregoing experiments demonstrate clearly that morphine, papaverin and benzyl alcohol have the same action upon the terrapin heart as upon smooth muscle. An overly cautious conclusion would go no further than this. But since the

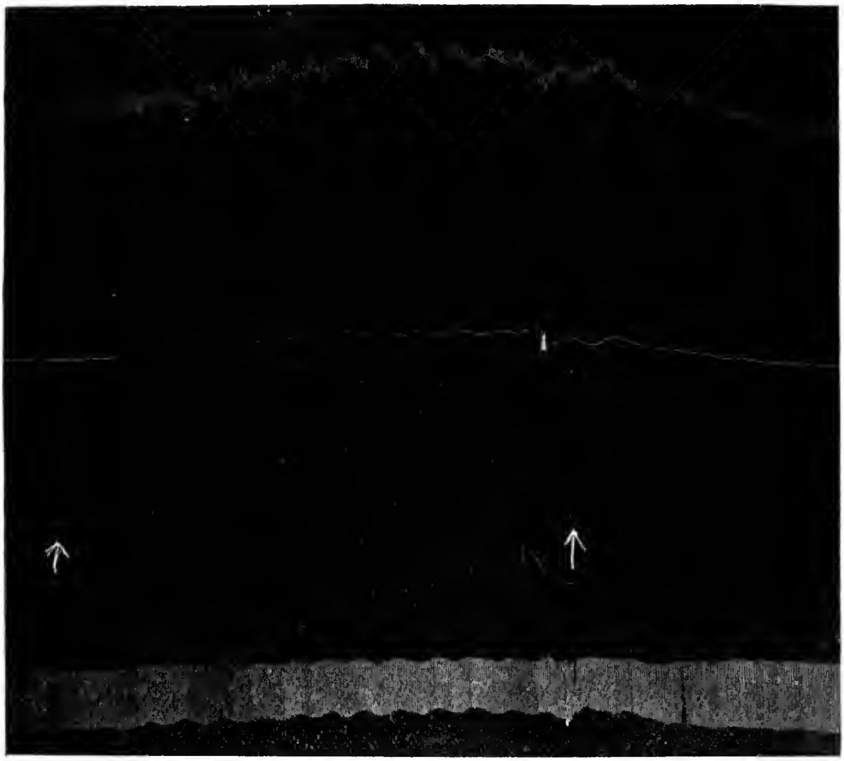


7.4 ↑ 7.6 ↑ Benzyl Alcohol 0.02% 7.6

FIG. 3. SHOWS THE PARALYSING EFFECT OF BENZYL ALCOHOL UPON THE TONUS OSCILLATIONS ELICITED BY A CHANGE FROM A P_H VALUE OF 7.4 TO 7.6

The hydrogen ion change was made at the first arrow, the benzyl alcohol was introduced at the second arrow.

smooth muscle is known to exist in the turtle's heart it requires no stretch of the imagination to see that the record made by these organs when the tonus waves appear may be made up of the ac-



7.4 ↑ Morphine c7.3 0.01% ↑ B. A. 0.01% c7.6

FIG. 4. SHOWS THE DOMINATING EFFECTS OF THE DRUGS OVER THE HYDROGEN ION INDEX

At the left hand (arrow) morphine brings on an increase of tonus in spite of the high concentration of H-ions. At the second arrow the introduction of benzyl alcohol inhibits the tonus although the perfusate has a lower concentration of H-ions.

tivity of two rhythmically beating tissues. The lower tonus waves obviously then would be the rhythmical smooth muscle contractions, and upon these are superposed the quicker beats of

the cross-striped cardiac tissue proper.[1] This analysis of the tonus waves on the drum records holds especially well for the auricular walls. It is also known that smooth fibers are present in the wall of the ventricle and our analysis ought to hold quite as well for that chamber. In our experiments, however, tonus waves in the auricles may cause alteration in tension filling and thus pseudo-tonus waves in the ventricles.

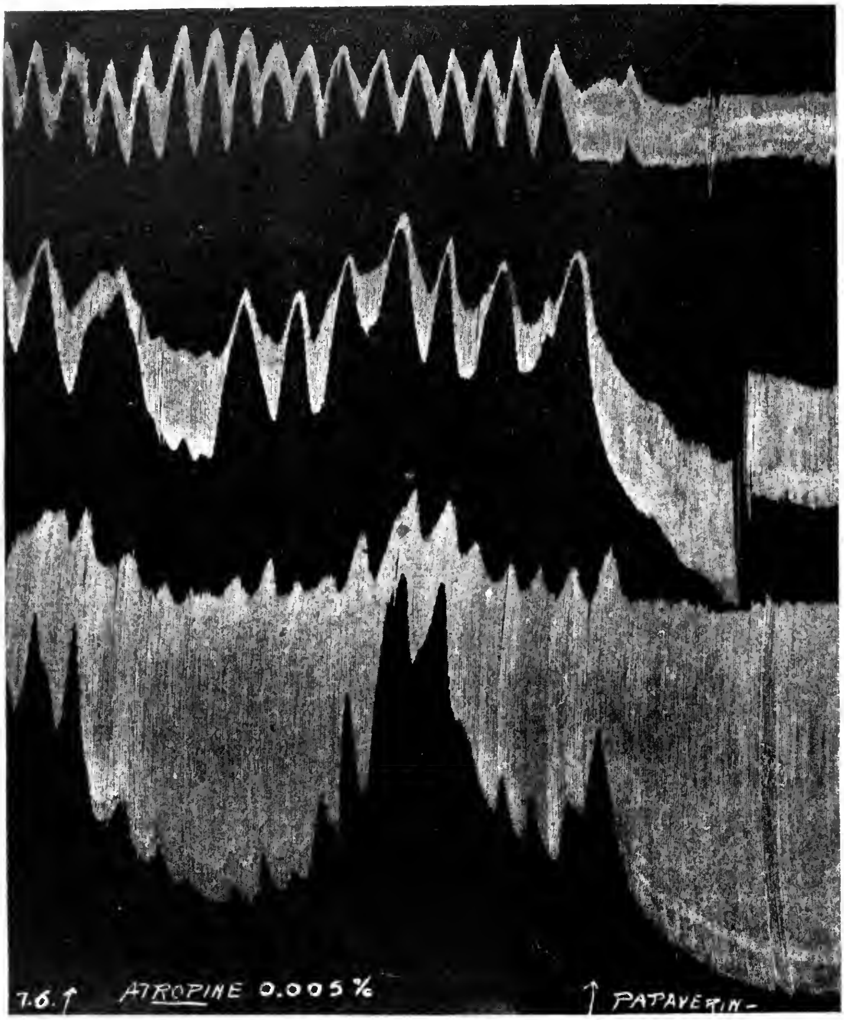
The question as to what part the endings or myoneural junctions of nerves played in the phenomena here studied was next taken up. The evidence of the effect of direct electrical stimulation of nerves is still conflicting (6). In the present investigation the answer was sought by the use of drugs. To paralyze the vagal nerve endings atropine was used.

A drum record of such an experiment is shown in figure 5. In this case all the heart chambers had been thrown into violent tonus waves by a Ringer of a P_H index of 7.6. Then atropine sulphate was added to the solution to make up a concentration of 0.005 per cent and passed through the heart for ten minutes. This produced little evident change. Evidently the low H-ion effect is on the muscle fiber directly or the positive tonotropic nerve fibers are sympathetic in origin.[2] At the second arrow in the figure the perfusate was changed to one containing papaverin hydrochlorid, 0.001 per cent. This resulted in a striking fall of tonus, inhibition of tonus waves and increase of amplitude of beat in all three chambers.

Similarly atropine failed to have any noticeable effect upon tonus waves that had been inaugurated by morphine. But benzyl alcohol, 0.002 per cent, following the administration of atropine inhibited all tonus waves and increased the amplitude of heart beats (see fig. 6). The effect of papaverin and of benzyl alcohol upon tonus and tonus waves appears therefore to be due to an action of the drugs upon the smooth muscle fibers directly rather than upon the endings of parasympathetic nerves.

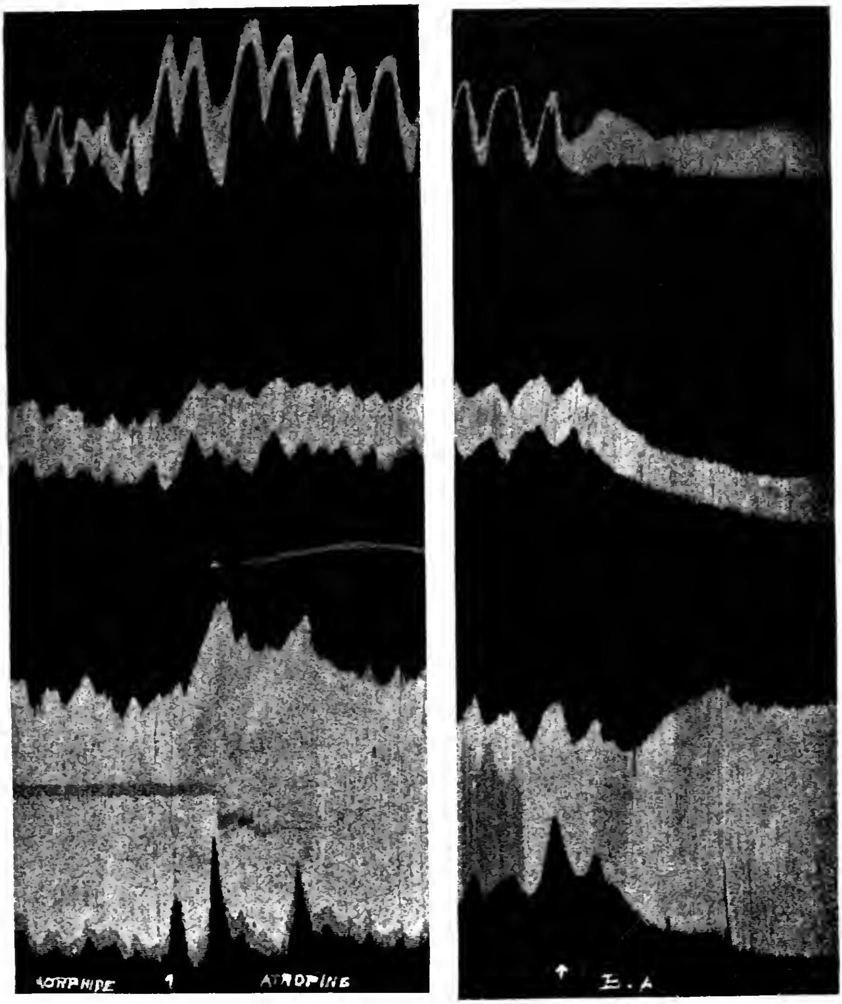
[1] It is quite unnecessary to invoke here the fibrillar-sarcoplasm theory of Bottazzi (5).

[2] Gruber and Markel (8) have shown that atropine increases the tonus of suspended strips of sino-auricular walls of terrapin heart. No control of the hydrogen-ion concentration was carried out however.



7.6 ↑ Atropine 0.005% c7.6 ↑ Papaverin 0.001% c7.6

FIG. 5. SHOWS THE TONUS INHIBITING ACTION OF PAPAVERIN (0.001 PER CENT) FOLLOWING THE ADMINISTRATION OF ATROPIN (0.005 PER CENT)



Morphine SO_4 ↑ 0.01% 7.3   Atropine SO_4 0.005% 7.6   ↑ B. A. 0.002% 7.6

FIG. 6. SHOWS THE FAILURE OF ATROPIN TO MODIFY THE TONUS WAVES CALLED FORTH BY MORPHINE IN THE LEFT HAND HALF OF THE FIGURE

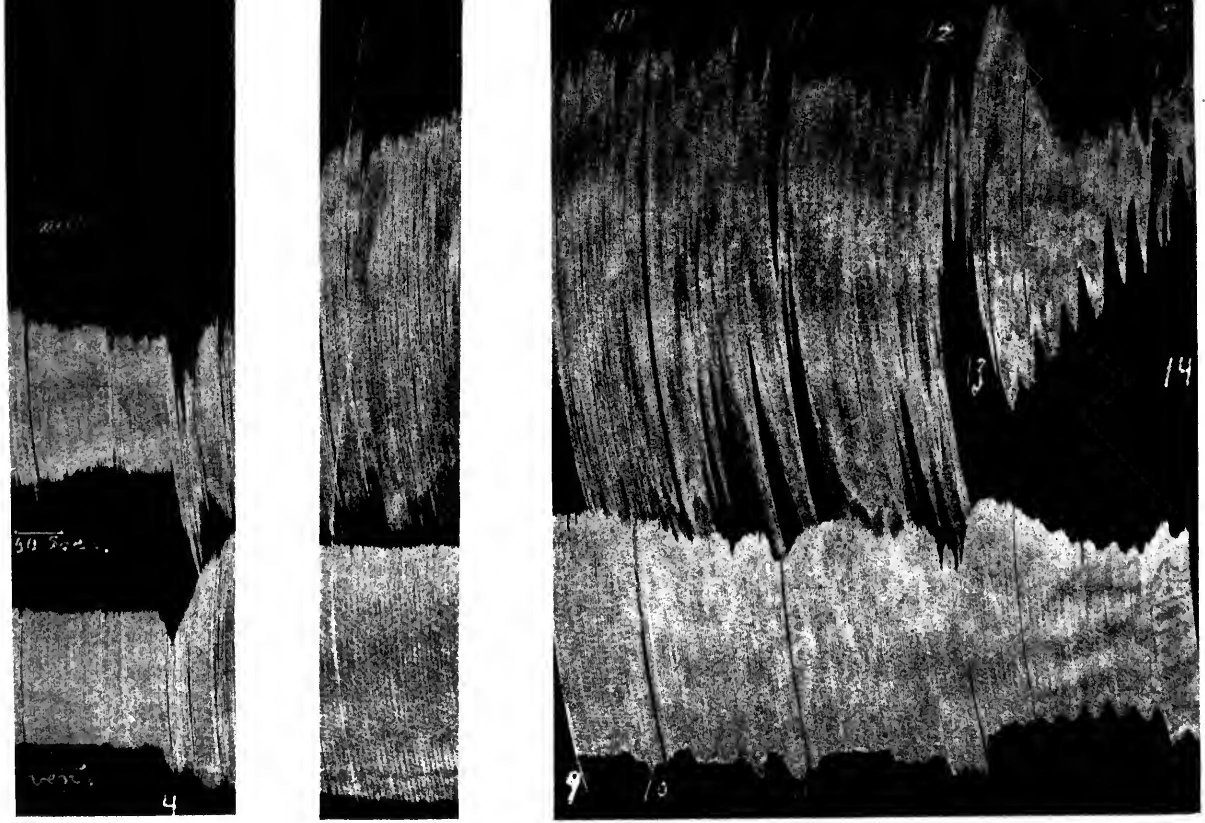
In the right hand half the same heart after an interval of ten minutes shows its prompt response to benzyl alcohol. 0.002 per cent in Ringer.

If we may regard epinephrine as a specific stimulant to the myoneural junction between sympathetic nerves and smooth muscle substance then its presence in solutions of various P_H index ought to yield significant results, however puzzling they may be in part. At the outset we must admit that if all the tonotropic fibers are sympathetic then, as in systemic arterial regions, we may get a positive or a negative tonus effect depending upon the concentration of the epinephrine on the one hand and the preponderance of the nerve supply of one set of fibers over the other or a difference of their junctural substances in sensibility to the drug.

Inasmuch, however, as epinephrine has a dilating effect upon other smooth muscle in the thoracic viscera (oesophagus, bronchioles, coronary artery), it may be explained that this effect is due to the stimulation of dilator or negative tonotropic fibers of sympathetic origin. If so then it would not be strange if the smooth muscle of the heart also would have a similar predominance of negative tonotropic fibers, responding to epinephrine in a manner similar to that of other thoracic viscera. Indeed such a result has already been observed by Gruber and Markel (7).

Our experiments with epinephrine were controlled rigorously throughout as to the hydrogen-ion concentration of their solutions. The results require description in considerable detail. The drum record reproduced on the accompanying folder as figure 7 should be followed from left to right together with the explanatory note to the figure.

From this record it appears, as from all the experiments, that if tonus waves are present with the P_H index of the perfusate at 7.3 or 7.65 addition of adrenalin hydrochlorid 2 or 3 parts to 10^7 has no other effects than to augment the tonus waves already prevailing. If the P_H index is lowered nearly to complete neutrality no increase of tonus is elicited, or tonus waves. That is, the heart goes on acting as if no adrenalin had been added. There may be some slight fall of tonus, if that previously had not been complete. This last point is brought out well in the record shown in figure 8.



$P_H = 7.1$ Do. + Adren., $8:10^7$ $P_H = 7.6$ $P_H = 7.1$ Adren., Do. $P_H = 7.4$

FIG. 8. UNTIL POINT *4* IN THE RECORD, NEARLY FIFTEEN MINUTES, THE HEART HAD BEEN PERFUSED WITH ADRENALIN-FREE RINGER OF A P_H INDEX = 7.1

From the point *4* on, the perfusate contained in addition adrenalin ($8:10^7$). Between the points *9* and *10* the heart is responding to benzyl alcohol in Ringer of 7.6 P_H index. This was followed by Ringer of identical P_H index but free of the alcohol, with resulting increase in tonus (sections *10* to *11*). Between the points *11* and *12* to a Ringer of 7.1 P_H index adrenalin of $8:10^7$ is perfused through the heart. Between *13* and *14* a plain Ringer of 7.4 P_H index is the perfusate.

In this record a tonus-free beat on the part of both auricle and ventricle is shown at the left, (P_H = 7.1). Addition of adrenalin ($8:10^7$) brings on no increase in tonus condition. The great effect on amplitude will be taken up later. Close inspection of the record shows some decrease of tonus, or an increase in diastolic relaxation. Again it is seen in this record (Fig. 8) that after tonus waves are induced on top of powerful beats by a perfusate of a high P_H index then adrenalin in a perfusate of a low P_H index does not increase tonus waves. The low reaction index of the adrenalin containing solution lowers the positive tonotropic irritability of the muscle substance. The stimulus of the adrenalin can not pass beyond the myoneural junction. For so soon as the P_H index of the solution, now free from adrenalin, is raised to no more than 7.4, prompt rise of tonus comes on. Enough adrenalin apparently is stored in the myoneural junctions to be effective just as soon as the irritability of the muscle substance itself is somewhat raised (see right half of figure 8). These combinations of adrenalin with low and high indices of the P_H value were repeated several times always with the same results as these records show.

The effects of epinephrine and atropine upon the terrapin heart under the conditions of the above experiments, the one promoting tonus, the other not interfering with it (in cases where it already exists), all point to a *sympathetic* origin for the *positive tonotropic* fibers.

The fact that under a condition of depressed tonus-irritability of muscle substance epinephrine may decrease tonus together with the fact that atropine does not induce a fall of tonus further points to the *sympathetic* as the origin of the *negative tonotropic* fibers.

The augmenting effect of epinephrine upon the strength of the heart beat is an old observation. Obviously low tonus of the smooth muscle in the heart wall is a *sine qua non* for the greatest amplitude of beat for the cross-striped part of the tissue. This seems to be the state prevailing for the left hand sections of figure 8. On the other hand addition of epinephrine to the perfusate of a heart already in maximal tonus (fig. 7) could not be expected

to increase amplitude of beat so long as the tonus maintaining constituent of the perfusate is present.

The action of epinephrine on the amplitude of beat suggests that the *positive inotropic* fibers to the heart are of *sympathetic* origin. The fact that pilocarpine decreases the amplitude (8) however indicates that the *negative inotropic* fibers have a *parasympathetic* origin.

CONCLUSIONS

1. The action of morphine, papaverin and benzyl alcohol on the isolated terrapin's heart is the same as upon smooth muscle structures. Morphine augments, papaverin and benzylalcohol depress tonus and tonus oscillations.

2. Since it is known that the action of these drugs upon smooth muscle is specific, and since it is further known that the chelonian heart is well supplied with smooth muscle besides the regular cross-striped cardiac type, it is concluded that the tonus effects of the drugs above mentioned on the terrapin heart are due to their action upon the smooth muscle portion of the heart walls.

3. Since the tonus augmentation brought on by a Ringer's solution of the lower hydrogen ion concentration is obliterated by the addition of papaverin or benzyl alcohol, and since these latter drugs depress the tonus of only smooth muscle, it is further concluded that the tonus effects of hydrogen ions upon the terrapin heart are likewise due to a direct action upon the smooth rather than the cross-striped portion of the musculature.

4. Experimental evidence is given to show that the action of papaverin and benzyl alcohol is on the muscle fibers directly and not upon the elements that are paralyzed by atropin.

5. The effect of epinephrine upon the terrapin heart is in part a function of the hydrogen-ion concentration of the perfusate. If the latter is of an order appropriate to maintain tonus just short of maximum then epinephrine will augment both tonus and tonus waves. If the tonus is already at a maximum the drug of course when added can have no further effect. If on the other hand the hydrogen-ion concentration is appropriate to bring on a condition of atonia in the heart walls epinephrine does not

elicit tonus or tonus waves, but increases the amplitude of the heart beats to a maximum.

This action of the epinephrine is explained on the basis that the hydrogen-ion concentration determines the tonus irritability of the muscle substance only, whereas the drug acts upon the myoneural junctions of the sympathetic nerve fibers. If the tonus of the muscle substance is positive then epinephrine will increase it, promoting tonus and tonus waves; if tonus in the muscle substance is negative, epinephrine will decrease it still further. These facts point to a sympathetic origin, as opposed to a parasympathetic origin, of both positive and negative tonotropic nerve fibers.

While the negative inotropic fibers to the heart are doubtless of parasympathetic origin, the action of epinephrine in these experiments points to a sympathetic origin for the positive inotropic fibers.

REFERENCES

(1) Andrus: Amer. Journ. of Physiology, 1919, xlviii, 221.
(2) Rosenzweig: Arch. f. Physiol. u. Anat., Physiol. Abt., Suppl. 1903, 206. Bottazzi e Ganfini: Zeitschr. f. allg. Physiologie, 1907, vi, 171.
(3) Pal: Deutsch. med. Wochenschr., 1913, 395.
(4) Macht: This Journal, 1918, xi, 389; Jour. Amer. Med. Assoc., 1915, lxiv, 1489.
(5) Bottazzi: Jour. of Physiol., 1897, xxi, 1.
(6) Fano et Sciolla: Arch. ital. de Biol., 1887, ix, 61; Bottazzi: Ibid., 1901, xxxvi, 277; Oinyma: Arch. f. d. gesammt. Physiol., 1910, cxxxiii, 500.
(7) Gruber and Markel: This Journal, 1918, xii, 43.
(8) Gruber and Markel: This Journal, 1918, xii, 53.

THE ACTION OF ADRENALIN ON THE HEART

I. ACTION ON THE TURTLE HEART

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Brown (1), Biedl and Reiner (2), Cybulski (3), and others have shown that adrenalin extract or solution of the desiccated gland, when injected into the cephalic end of the carotid artery of dogs, produces a distinct slowing of the heart previous to the rise in pressure. Many other experimenters (Brown, Nice, Rock and Courtwright (4)) mention the respiratory acceleration after adrenalin, and seem to think it due to direct central action. As a result of our work with turtles we have come to a similar conclusion, that adrenalin, at least in this animal exerts a direct action on the vagus centers.

In this investigatiom it was our object to obtain evidence of the direct action of adrenalin on the medullary centers. To do this we resorted to brain perfusion as the most simple and reliable method. We prepared our turtles by cutting the cord low enough to preserve the medulla, and usually between the third and fourth cervical vertebrae. In order to reduce the number of spontaneous movements to a minimum, the cord was pithed. Later we found it advisable to break the femurs and humeri and cut the abdominal muscles in order to abolish all movements.

The two carotid sheaths and their contents were carefully dissected out as high up in the neck as possible in order that the drug may more quickly reach the medulla, and also that less resistance would be offered by any vaso-constriction. The vagi nerves were isolated and subjected to stimulation in order to determine their effectiveness in producing inhibition. We found the right considerably more effective and sensitive than the left vagus.

It was not thought necessary to tie off all the heart vessels so long as none of the drug was allowed to enter the general circulation and hence exert a direct action on the heart. It is evident that the heart, when all the large vessels are tied off, as is usually done in perfusing the brain, may develop an abnormal beat and hence would not in all probability respond in a normal manner to central stimulation. We were concerned, therefore, with retaining as near a normal beat as possible. To effect this a ligature was passed around the neck, excluding the two carotid sheaths and their contents, and tightly drawn between the third and fourth cervical vertebrae. Opening a few veins amply takes care of the excess fluid. In this manner the head is practically isolated from the body, but the heart is still under the influence of the vagus centers through the vagi nerves..

A cannula was inserted into the right carotid and in our first experiments the left was tied off; but in our later ones, to exclude the probable effect of anemia at least in a large degree, the left was not occluded. The solutions used—1: 50,000 adrenalin, 1–10,000 strychnine sulphate, and 0.9 per cent salt solution and 1: 100,000—adrenalin were run in from bottles under such a pressure that perfusion was possible, but that the liquid did not back up into the left carotid and hence act directly on the heart.

In a few cases adrenalin was injected directly into the heart muscle with the same results as obtained by many investigators: —namely, that the amplitude and rate are increased, adrenalin acting as a stimulant.

Experiment I

The normal heart rate for this animal, under the conditions of this experiment, was 29 per minute and after perfusion with salt solution it increased to 31. A 1–50,000 adrenalin solution was next used and in three minutes and ten seconds the heart was totally and abruptly inhibited (see fig. 1). There was no gradual slowing for just previous to the inhibition the water was 31. Normal salt solution was then used and in a somewhat general manner the heart rate returned to 33. It took about two minutes after the salt perfusion for the heart to return to normal.

A 1–10,000 strychnine sulphate solution was next used with the idea that it might sensitize the cells of the vagus centers that they be more easily influenced by adrenalin and hence produce aggravated results. Partial inhibition was effected by the strychnine, the heart rate dropping to 4 (see fig. 2). Just preceding this slowing the rate increased to 39. A 1–50,000 adrenalin solution was next run in and in five minutes complete inhibition was again effected. It seems rather improbable that strychnine sensitized the nerve cells in this animal, but probably stimulated them to some extent.

With a return to salt solution the heart rate increased to 38, but gradually fell to normal, 31. A 1–100,000 solution of adrenalin was used and partial inhibition was effected, the rate dropping to 12 in one minute. However, there was a gradual return to normal due in all probability to a stoppage in the circulation of the fluid (see fig. 3).

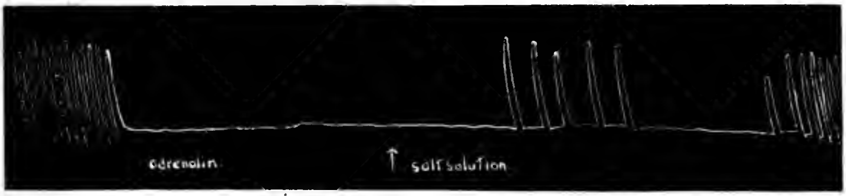

Fig. 1. Total Inhibition of Turtle Heart Following Perfusion of Brain With 1–50,000 Adrenalin

We have found it impossible to effect these results for any length of time after perfusion has been started. There are three possible causes for this; (1) anemia; (2) tolerance being established; (3) fatigue of the center. It is well known that nerve cells suffer readily from lack of nutrition and, since in this experiment no nourishment was being brought to the centers, one is justified in assuming that the nerve cells failed to react to the adrenalin stimulation because of the deprivation of metabolic material. It is possible that tolerance was established for it has been found that continued use of some drugs produces a tolerance for those drugs. Although we have no definite means as yet of differentiating between the tolerance and fatigue in the turtle under the conditions of this experiment we feel justified in saying that tolerance may be a factor worthy of consideration.

Fig. 2. Partial Inhibition Effected by Perfusing the Turtle Brain With 1–10,000 Strychnine Sulphate Solution

Normal salt solution substituted at arrow

Fig. 3. Partial Inhibition Effected by the Perfusion of 1–100,000 Adrenalin Through Turtle's Brain

Perfusion started at the arrow. Note return to normal rate due probably to anemia

Fatigue of the center is the third probability, but due to an absolute anemia being produced it is quite impossible to determine whether fatigue is a factor under the present conditions. We, ourselves, feel that the anemia is responsible for the greater part, but do not cast aside fatigue and tolerance as unimportant.

TABLE 1

HEART RATE	REMARKS
29	Normal
31	After salt perfusion
Inhibition	After 1-50,000 adrenalin sol.
33	After salt perfusion
4–9	After strychnine 1-10,000
39	After salt solution
Inhibition	After 1-50,000 adrenalin
38–31	After salt solution
12	After 1-100,000 adrenalin
37	After salt solution

Experiment II

In experiment I, distilled water was used as a solvent. It may be assumed that this fluid, by producing osmotic changes and anemia might exert some influence on the heart rate. In order to disprove this we perfused the brain of this turtle with distilled water. Inhibition was not produced, but following this procedure adrenalin inhibition could not be effected due in all probability to the production of absolute anemia by the distilled water. A 1: 1,000 adrenalin solution (1 cc.) was injected directly into the heart muscle. The amplitude of this beat was increased enormously, 75 to 100 per cent. Accelerations was also effected. Since the sympathetics to the heart in this animal are less functionally developed than in mammals, we should not expect any increase in rate from stimulation of the myoneural junctions. We base this statement on the fact that the vagus is not well developed in the turtle as in mammals, for a stronger stimulus is necessary to elicit inhibition of the turtle heart than of the dog heart, and also because the left vagus is in a majority of the cases irresponsive to stimulation; and hence there is no reason to assume that the sympathetic system, which is of later development and which requires even a stronger stimulus than does the vagus to produce proportional results, is as well devel-

oped functionally as the vagus. We may conclude from this indirect evidence that adrenalin exerts a direct influence on the heart muscle (fig. 4).

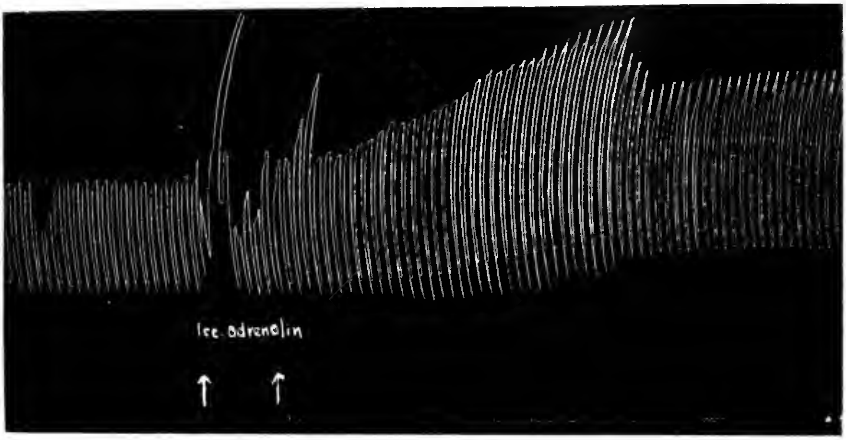

FIG. 4 ONE CUBIC CENTIMETER OF 1–1000 ADRENALIN WAS INJECTED DIRECTLY INTO THE HEART MUSCLE

Experiment III

The routine of this experiment was the same as in the above two with the exception that 0.9 per cent salt solution was used as a solvent. The left carotid artery was left open so that the brain received a partial supply of blood at least. The pressure of the inflow was such that perfusion was possible but still not great enough to force the liquids back down the open left carotid artery.

The normal rate was 38. Adrenalin 1–50,000 produced total inhibition in one-half minute. With salt solution alone the heart rate became 43, but with the return of adrenalin total inhibition resulted in one minute. Perfusion with salt solution caused the heart to beat at 40.

The promptness with which the centers responded to adrenalin solution in this experiment, we think is due at least in a degree to the better nutrition of the cells. Strychnine was not used because of its central inhibiting action.

TABLE 2

HEART RATE	REMARKS
38	Normal
Inhibition	After 1–50,000 adrenalin
43	After salt solution
Inhibition	After 1-50,000 adrenalin
40	After salt solution

Experiment IV

The technique of this experiment was the same as that of three. The normal heart rate was 40. After perfusion with salt solution for about five minutes the rate was 43. 1–50,000 adrenalin was then used, total inhibition resulting in 4 minutes. With return of salt solution the heart rate became 38.

TABLE 3

HEART RATE	REMARKS
40	Normal
43	After salt solution
Inhibition	After 1-50,000 adrenalin
38	After salt

Experiment V

The normal rate of this turtle heart was comparatively slow, normal being 19. Perfusion with salt solution effected no changes. After adrenalin, however, the rate dropped quickly to 6; and with the return of salt solution a normal rate was assumed. Adrenalin perfused through the cerebral vessels a second time slowed the heart to 5. Salt solution

TABLE 4

HEART RATE	REMARKS
19	Normal
19	After salt solution
6	After adrenalin
18	After salt solution
5	After adrenalin
18	After salt solution
8	After adrenalin

again effected a normal rate, and adrenalin the third time slowed the heart to 8. Complete inhibition was not effected. An extremely strong current applied to the right vagus was necessary to slow the heart. The left vagus did not respond to stimulation. Anemia or tolerance was produced for perfuson a fourth time with adrenalin elicited no changes at all.

In all we used nine turtles with identical results except in turtle II when an absolute anemia probably resulted from the perfusion with distilled water.

CONCLUSIONS

From these experiments we may conclude that:—

1. Adrenalin exerts a direct action on the cardio inhibitory center of the turtle stimulating it and hence producing stoppage of the heart.
2. Due to central anemia, fatigue, or tolerance being established adrenalin exerts no action after repeated use.
3. Adrenalin when injected directly into the heart muscle acts as a stimulant, increasing both rate and amplitude, systole being most affected.
4. Strychnine exerts some action on the medulla which produces partial inhibition. (This needs further investigation).
5. Adrenalin is not any more efficacious after strychnine than before.

REFERENCES

(1) Brown: Journ. Pharm. & Exper. Therap., 1916, viii, 1915.
(2) Biedl and Reiner: Archiv. für die ges. Phys., lxxiii, 385.
(3) Cybulski: Centralbl. f. Physiol., 1895, 172.
(4) Nice, Rock and Courtwright: Am. Journ. Phys., 1914, xxxiv, 326.

THE SALICYLATES

XI. THE STABILITY AND DESTRUCTION OF THE SALICYL GROUP UNDER BIOLOGICAL CONDITIONS

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I. INTRODUCTION

In connection with previous work on the salicylates it has been regularly observed that the solutions of sodium salicylate and other salicyl compounds gradually lose in strength on standing. This is a fact not generally appreciated, owing to the prevailing impression that phenolic compounds are quite stable. The results of the older investigators gave the impression that the salicyl group is practically unchanged in the body. Previously reported data from this laboratory, however, have shown that 20 to 30 per cent of the salicyl in its passage through the body remains unaccounted for, and is presumably destroyed (1). The data here presented, indicate the relative ease with

which salicyl is decomposed under ordinary conditions, and lend support to the previous contention that the salicyl unaccounted for in the excretion experiments is actually destroyed by the organism.

The compounds studied were principally sodium salicylate and to some extent acetyl-salicylic acid, (aspirin), salicylo-salicylic acid, (diplosal) and methylene citricyl salicylic acid (novaspirin).

II. RESULTS

1. Deterioration of solutions of salicyl compounds

The solutions of different salicylates in water and alcohol were allowed to stand in sunlight, and in the dark under ordinary laboratory conditions. The salicyl content was estimated colorimetrically from time to time, according to the technique previously described by Thoburn and Hanzlik (2).

The results presented in table 1 illustrate the loss in salicyl content of different salicyl compounds on standing.

It is seen that the greatest deterioration is exhibited by sodium salicylate, less by salicylo-salicylic acid and least by acetylsalicylic acid. This is probably due to the fact that acetylsalicylic acid and salicylosalicylic acid are slightly acid in reaction, tending to inhibit fungus and bacterial growths, and chiefly because they were in solution in alcohol, which acts as a preservative. Sodium salicylate was dissolved in water. When chloroform was added as preservative, the solution remained unchanged almost indefinitely. It has now become a routine measure in the laboratory to add chloroform to our standards of sodium salicylate, which are weak solutions, used in quantitative estimations.

All salicyl solutions on standing exhibit considerable fungus growths, while, if these are prevented by the addition of chloroform, the solutions remain clear and chemically unchanged. It appears therefore that deterioration of aqueous solutions of sodium salicylate, at least, is due to the presence of living matter (fungi, etc.).

TABLE 1

Deterioration of salicyl solutions on standing

COMPOUND USED	STRENGTH SOLUTION USED	TREATMENT	MILLIGRAM IN 1 CC. FRESH SOLUTION	MILLIGRAM IN 1 CC. AFTER STANDING	PER CENT SALICYL LOST END OF
Sodium salicylate	1 per cent in water	Stood at room temperature	8.5	6.25	21 (4 months)
Acetylsalicylic acid (aspirin)	1 per cent in alcohol	Stood at room temperature	7.5*	7.06*	5 (4 months)
Salicylosalicylic acid (diplosal)	1 per cent in alcohol	Stood at room temperature	10.0*	8.75*	11 (4 months)
Methylene citricyl salicylic acid (novaspirin)	1 per cent in alcohol	Stood at room temperature	5.0*	5.0*	0 (4 months)
Sodium salicylate	0.012 per cent in water	Stood at room temperature	0.1	0.080	20 (68 days)
Sodium salicylate	0.012 per cent in water	Stood at room temperature	0.1	0.068	32 (86 days)
Sodium salicylate	0.012 per cent in water	Stood at room temperature	0.1	0.066	34 (105 days)
Sodium salicylate	0.012 per cent in water	Stood at room temperature	0.1	0.066	34 (135 days)
Sodium salicylate	0.012 per cent in water	Stood at room temperature	0.1	0.060	40 (159 days)
Sodium salicylate	0.012 per cent in water	Stood at room temperature	0.1	0.060	40 (192 days)
Sodium salicylate	0.012 per cent in water	Stood at room temperature	0.1	0.046	54 (272 days)

* Assayed by previous hydrolysis with sodium hydroxide and subsequent distillation with phosphoric acid and colorimetric estimation of the distillate. The differences are not due to the distillation and hydrolysis, since, with 0.75 mgm. and 7.8 mgm. quantities of sodium salicylate by direct colorimetric estimation alone and after hydrolysis and distillation of the same, the results were 0.75 mgm. and 7.2 mgm, respectively.

The remaining assays were made by direct colorimetric estimation.

2. Destruction of salicyl by yeast fungus

In order to furnish more evidence for the destruction by lower organisms the matter was further tested by treatment of solutions of sodium salicylate in water with yeast under various conditions. Aqueous solutions of known concentration of sodium salicylate were allowed to stand with yeast at room temperature

and also incubated at 37.5°C. with and without chloroform, in glass stoppered flasks, and the concentrations determined from time to time. The results are presented in table 2. These indicate that salicylate in the presence of yeast deteriorates to a lesser extent than when allowed to stand alone. The viability of the yeast at the end of the experiments was not determined. The only two solutions which underwent considerable decomposition (28 per cent and 36 per cent) were the controls without yeast and chloroform and these contained many strands of some kind of fungi, which, however, were not identified. The solutions containing liberal quantities of chloroform retained their strengths practically unimpaired at the end of eight months.

TABLE 2

Effect of yeast fungi on sodium salicylate

ORIGINAL STRENGTH OF SODIUM SALICYLATE IN WATER	TREATMENT	STRENGTH AT END OF 2 MONTHS	STRENGTH AT END OF 8 MONTHS	LOSS
per cent		*per cent*	*per cent*	*per cent*
0.25	Stood at room temperature (without yeast)	0.23	0.16	36
0.25	Contained liberal quantity of chloroform at room temperature	0.25	0.25	None
0.25	Incubated with yeast at 37.5°	0.20	0.208	16
0.25	At 37.5° (without yeast)	0.20	0.18	28
0.25	Contained liberal quantity of chloroform at 37.5°	0.25	0.25	None
0.25	Yeast together with very small quantity of chloroform	0.23	0.208	16

3. *Recovery of salicylates from animal organs*

The results obtained with standing salicylate solutions (developing fungi) and yeast naturally led to experiments with organs of animals. Dixon (3) states that salicylate is chemically changed by the liver. However, we have not been able to locate the evidence supporting this statement. As a preliminary step incubations of salicylate with freshly obtained and hashed organs of dog were tried. The organs of dog were used since the uri-

nary excretion with dogs is less than with human individuals, indicating a greater destruction. Four cubic centimeters of a 1 per cent. solution of sodium salicylate were added to 50 grams of hashed muscle, liver, brain, pancreas, intestine and blood. One set of preparations was covered with liquid petrolatum (absence of oxygen) the other set was left exposed (presence of oxygen). At the end of seventy-two hours, the mixtures were extracted with 95 per cent alcohol, filtered, washed and the salicylate in the filtrates determined by distillation and colorimetric estimation in the usual way. The results are presented in table 3.

TABLE 3

*Percentage recovery of salicylate after treatment with different organs**

ORGAN	SALICYL RECOVERED	
	Exposed	Covered with liquid petrolatum
	per cent	*per cent*
Muscle	62.5	41.4
Liver	46.0	51.4
Brain	44.4	56.0
Pancreas	61.6	
Intestine	56.0	58.4
Blood	47.2	50.0

* Fifty grams of each of the organs and 4 cc. of 1 per cent sodium salicylate were used.

These results indicate no special differences in the behavior of the different organs. The small differences which occur are well within the range of experimental error. The relatively small recoveries may be due to retention of the salicyl by adsorption, chemical combination or actual destruction.

That the salicyl group is not entirely retained by adsorption and chemical combination is indicated by the results from a series of 28 experiments presented in table 4 and carried out with different salicyl compounds added to liver under different conditions. That is, small quantities of the different salicyl compounds such as would be encountered after therapeutic doses of these were added to fresh ground liver of different dogs, and after standing from about four to twelve hours the preparations were

extracted with saline and strong alcohol. The solvents were used in the cold, with heat on water-bath, and after preliminary digestion of the liver with concentrated phosphoric acid, and in single and double volume.

The extracts were filtered and an aliquot (two-thirds of the entire volume) portion of the filtrate was used for recovery and estimation of salicyl in the usual way after preliminary removal of the alcohol by distillation in those experiments in which alcohol was used.

The results (table 4) show that extraction with alcohol and heat gave the highest recoveries and these were only somewhat enhanced when double the volume of alcohol was used.

Saline extraction yielded the lowest results, which may be due to the poor solvent qualities of water, or greater destruction of salicyl, while alcohol is a better solvent and also inhibits cellular activity, permitting in this way greater recoveries as was found to be the case. Preliminary destruction of the tissue by concentrated phosphoric acid did not liberate any more salicyl than alcohol alone without the acid. The different salicyl compounds gave about equal recoveries considering the experimental error under the conditions. Considerable difficulty was encountered with the appearance of fatty acids in the distillates. However, removal of these from the distillates by ether extraction did not improve the estimation as compared with the direct colorimetric estimation in their presence.

In another series of experiments the organ residues were thoroughly washed after filtration and subjected to destructive distillation with concentrated phosphoric acid. The total recoveries were still low, indicating that the salicylate was destroyed.

Taking everything into consideration it appears that the salicyl group undergoes destruction when added to hashed organs (liver, etc.). However, owing to the difficulties of satisfactorily recovering salicyl from tissues a final decision as to this is impossible at present.

The study in this direction was not pursued further, since the final proof required is that salicyl is actually destroyed by the

TABLE 4

Recovery of salicylates from dog's liver under different conditions

NUMBER OF EXPERIMENT	SALICYL COMPOUND	ADDED TO LIVER	WEIGHT OF LIVER USED	SALICYL RECOVERED	REMARKS
Extraction with saline (0.9 per cent NaCl) in cold, using 450 cc.					
		mgm.	*grams*	*per cent*	
31	Sodium salicylate	17.2	75	27	
32	Sodium salicylate	34.4	75	35	
33	Acetylsalicylic acid	15.4	75	28	
34	Acetylsalicylic acid	30.8	75	40	
35	Salicylosalicylic acid	42.4	75	28	
Extraction with methyl alcohol in cold, using 450 cc.					
19	Salicylosalicylic acid	10.6	75	39	
20	Acetylsalicylic acid	17.7	75	20	
Extraction with methyl alcohol and heat, using 450 cc.					
14	Acetylsalicylic acid	7.7	50	75	
15	Sodium salicylate	8.6	50	58	
Extraction with ethyl alcohol and heat, using 450 cc.					
21	Sodium salicylate	17.2	75	57	
22	Salicylosalicylic acid	21.2	75	53	
23	Acetylsalicylic acid	15.4	75	53	
24	Sodium salicylate	34.4	75	66	
25	Acetylsalicylic acid	30.8	75	50	
Preliminary digestion with concentrated phosphoric acid, extraction with ethyl alcohol and heat, using 450 cc.					
36	Sodium salicylate	17.2	75	44.1	Control; without acid digestion.
37	Sodium salicylate	17.2	75	52.9	With acid digestion
38	Acetylsalicylic acid	15.4	75	62	Control; without acid digestion.
39	Acetylsalicylic acid	15.4	75	50	With acid.
Extraction with ethyl alcohol in cold, using 900 cc. (double volume)					
41	Sodium salicylate	12.5	75	60	
42	Methyl salicylate	18.1	75	60	
43	Acetylsalicylic acid	14.2	75	65	
44	Salicylosalicylic acid	11.5	75	46	

TABLE 4—*Continued*

NUMBER OF EXPERIMENT	SALICYL COMPOUND	ADDED TO LIVER	WEIGHT OF LIVER USED	SALICYL RECOVERED	REMARKS
Extraction with ethyl alcohol and heat, using 900 cc. (double volume)					
		mgm.	*grams*	*per cent*	
46	Salicylosalicylic acid	11.5	75	50	
47	Methylene-citricyl salicylic acid	10.0	75	18	
48	Sodium salicylate	14.4	75	78	
49	Sodium salicylate	14.4	75	76	
50	Acetylsalicylic acid	14.8	75	77	
51	Salicylosalicylic acid	11.5	75	39	

intact liver. For this reason perfusion of the liver which is known to be unsatisfactory technically, also was not attempted, but rather the excretion in urine was studied quantitatively in animals in which various conditions which might be expected to increase or diminish destruction were produced. Certain abnormal metabolic conditions in human individuals were also studied.

4. Destruction of salicyl by normal human individuals and in different diseased conditions

The calorimetric studies of Dubois (4) and associates show definitely that katabolism is increased in febrile conditions and in Basedow's disease (hyperthyroidism). With this in view quantitative excretion experiments with salicylates were carried out on human individuals in different conditions, controlling these as much as possible by comparison with the excretion from a number of apparently normal individuals. The evidence obtained, it is believed, supports the contention that salicylate is destroyed by the living organism. It is realized that evidence of this sort is valuable only when overwhelmingly convincing. It is intended, therefore, as a part of the collective evidence here offered. The variability in excretion with certain diseased conditions might throw light on the site or nature of destruction such, for instance, as the liver. Finally this could be controlled in animals, although apparently this is not as simple as it appears as indicated by the data obtained by us and also others.

A portion of the data has been previously reported (1) in connection with studies on excretion. The experiments were conducted as quantitatively as possible and in practically the same manner as before. That is, definite quantities of salicylate to "toxicity" were administrated by mouth, and the urine collected until salicyl-free. The salicyl was recovered quantitatively by the distillation-colorimetric method previously described. The various patients used for these particular experiments were selected after careful examination by experienced diagnosticians[1] continually in attendance at the hospital, and then were confined in a special ward for our purpose. The results obtained are presented in table 5.

a. Normal individuals (controls). As indicated by the results summarized in table 5, excretion of salicylate by apparently normal individuals was about 80 per cent (79.7 per cent) of the total administered. In a previous communication (1) it was shown that only an insignificant fraction of the remainder appeared in the sweat and feces. Therefore, about 20 per cent of the drug is apparently destroyed by the organism. None is retained since the urine was collected in each case until salicyl-free.

b. Excretion in fever. If one of the metabolic functions is to destroy salicyl and similar phenolic compounds, then an increase in metabolism such as is known to occur in fever would tend to further reduce the excretion as compared with normal individuals. This was found to be the case. The results in table 5 indicate a further loss of about 20 per cent in febrile conditions (principally rheumatic fever, and tuberculosis). This implies that the destruction is not specifically concerned with any particular organ, but takes place in all organs throughout the body, the increased destruction in fevers being simply a quantitative difference.

c. Excretion in nephritis. If destruction of salicylate is merely the expression of a general metabolic function, then retention of the salicyl as in nephritis with diminished renal functional efficiency would expose the drug longer to the destructive action of

[1] We wish to express our thanks to Drs. R. W. Scott and E. P. Carter of the City Hospital for their kind coöperation in this work.

TABLE 5

*Excretion of salicyl in normal individuals and different diseased conditions**

NUMBER AND INDIVIDUAL	DIAGNOSIS	QUANTITY SALICYL¶ ADMINISTERED	SALICYL EXCRETED	REMARKS
		Normal (controls)		
		grams	*per cent*	
6(F. D.)	Normal	12.04	91.9	
7(L. E.)	Tabes	13.76	72.4	At time of experiment patient was afebrile and free from symptoms of syphilis
8(M. H.)	Normal	15.48	77.3	
9(H. R.)	Normal	14.96	71.7	
21b(J. V.)	Normal	9.5	72.2	
P. J. H.	Normal	5.7	78.1	
26(R. H.)	Corneal ulcer	12.6	75.4	
27(J. M.)	Varicose ulcer	12.6	87.4	
28(J. S.)	Gastritis	8.0	82.8	Afebrile; gastritis
29(L. K.)	Recovered from rheumatism	8.9	87.5	Afebrile
30(I. M.)	Chronic arthritis	14.24	79.7	Afebrile
Median			79.7	
		Febrile		
1(P. F.)	R. F.	13.41	45.0	
2(J. M.)	R. F.	12.81	71.7	Moderate fever.
3(W. C.)	R. F.	15.48	73.7	Moderate fever
4(J. H.)	R. F.	14.45	59.5	
5 (J. L.)	R. F.	14.45	61.3	
11(S. V.)	R. F.	17.00	54.9	
12(W. B.)	R. F.	8 75	65.0	
21a(J. V.)	R. F.	10.20	57.7	
19(Y. V.)	T. B.	10.8	52.9	Very febrile
Median			59.5	
		Nephritis		
16(M. C.)	Chronic nephritis	(a) 12.3 (b) 11.4 (c) 10.5 (d) 9.36	63.7 55.1 53.6 60.4	Markedly diminished renal functional efficiency
34(K)	Nephritis	13.06	54.5	Markedly diminished renal functional efficiency
35(H. S.)	Chronic nephritis	5.87	64.7	Markedly diminished renal functional efficiency
Median			57.5	

TABLE 5—*Continued*

NUMBER AND INDIVIDUAL	DIAGNOSIS	QUANTITY SALICYL¶ ADMINISTERED	SALICYL EX-CRETED	REMARKS
		Drug habituation		
		grams	*per cent*	
10(J. B.)	Chronic alcoholism	19.3	45.8	
17(P. K.)	Chronic alcoholism	12.3	63.1	Afebrile
20(G. M.)	Chronic morphinism	10.8	63.2	
Median			63.1	
		Liver conditions		
31(E)	Hepatic abscess	11.5	65.0	Lues and febrile
32(P. K.)	Syphilis of liver	14.4	76.9	
33(F.)	Hepatic cirrhosis	9.0	87.4	
Median			76.9	
		Hyperthyroidism, etc.		
37(B.)	Basedow's disease	3.1	51.7	Severe attack
37(B)	Recovery	4.23	70.4	Recovered; about well 10 days later
37(B)	Basedow's disease	2.7	60.3	Recurrence after 5 months
36(F)	Gout	9.0	69.1	Fourteeen days later, after 9 grams = 78.8 per cent salicyl excreted

* R. F.=Rheumatic Fever; T. B =Tuberculosis.
¶ Expressed as salicylic acid.

the tissues resulting in diminished excretion. The results in table 5 tend to confirm this view. This was even more strikingly shown by dog 18 (see table 6) suffering with a severe acute nephritis produced by salicylate itself. This animal excreted only about one-half (26.6 per cent) of the salicylate that normal animals excreted (56.6 per cent).

d. Excretion by drug habitués. Habituation to morphine and alcohol is claimed by some to be due to an acquired power of the organism for increased destruction of these drugs. Two individuals habituated to alcohol and one to morphine were found to excrete much less (about 40 per cent) salicyl than normal individuals, indicating a greater destruction which agrees with the tendency in febrile and nephritic individuals.

e. Excretion in Basedow's disease. The increased katabolism in this condition should favor the destruction of salicyl. A young woman suffering with a severe form of Basedow's disease was subjected to the test three different times: (1) when the condition was most acute, the excretion was found to be 51.7 per cent; (2) about ten days later, when considerable recovery was apparent, the excretion rose to 70 per cent, and (3) during a recurrence of the condition five months later to about the original degree of severity, the excretion was only 60 per cent. The dosage on these different occasions remained practically the same and could not account for the differences. It is considered, therefore, that the changes in excretion coincided remarkably with the changes in the condition (altered metabolism). That is, a considerable destruction of salicyl occurred when the condition was most acute; a lessened destruction during the recovery period, which agrees, in general, with the tendency in normal individuals, and a considerable increase in destruction again when the condition recurred. At the time of the recurrence the patient was greatly emaciated, apprehensive, excitable and in every respect exhibited the symptoms of "hyperthyroidism" to a most pronounced degree. Unfortunately, further studies on excretion of salicyl in Basedow's disease have been impossible owing to lack of clinical material of this type in the City Hospital of Cleveland. The particular case reported, however, is so striking that it is believed to be confirmative of the tendency of the results described in this section, namely, an increased power for destruction of salicyl, and more pronounced than in normal individuals because of the increased metabolism.

f. Excretion in hepatic conditions. It is conceivable that the destruction of salicyl is the special function of some organ, although the results thus far presented do not indicate this to be the case. According to Dixon (2) the liver is responsible for the change. If this is true, then in diseased conditions in which the functional efficiency of the organ is reduced or abolished the excretion of salicyl should be greater than normal. Owing to the unreliability of liver function tests the proposition is admittedly difficult to test satisfactorily, and it is known that even a small

portion of a degenerated liver may carry on its function apparently unimpaired.

Nevertheless excretion was studied in three patients in whom it was felt beyond doubt by the clinicians that the liver was injured. The results were disappointing. Patient 31, who was also febrile, excreted only 65 per cent of the salicylate, while the other two patients (32 and 33) excreted 76.9 per cent and 87.4 per cent, respectively. That is, the results from patients 32 and 33 are within the range of excretion by normal individuals and can not be said to convey anything indicative of the indispensability of the liver for destruction of the salicylate. In patient 31 the low excretion may be due to the fever. Experiments on animals, in which degeneration of the liver was produced by phosphorus and to be referred to later, were also unsatisfactory in showing that the liver is specially concerned in the destruction.

5. Destruction of salicyl in animals

The object of these experiments was to control if possible the observations made on human individuals in different diseased conditions with the hope of explaining more definitely the site and nature of destruction of the salicylate. All observations were made in a quantitative manner. The dosage of salicyl per kilo corresponded to that used in human individuals. The salicyl was administered intramuscularly, intraperitoneally, hypodermically and into the stomach. When vomiting occurred the vomitus was carefully collected, the salicyl content estimated and deducted from the total administered. The urine was collected until salicyl-free, taking care to remove all cage contents; and estimated in the usual way by the distillation-colorimetric method.

Degeneration of the liver was produced by injections of yellow phosphorus dissolved in olive oil. The livers were examined microscopically by Dr. Maurice L. Richardson of the Laboratory of Pathology. In the two animals reported the livers were found to be markedly degenerated. Out of seven animals tried only two survived long enough for completion of the salicyl excretion in a satisfactory manner.

Thyroid administration was carried out with the hope of increasing metabolism and confirming the diminished excretion of salicyl observed in Basedow's disease in the fore-part of the paper. Carlson, Rooks and McKie (5) had previously found that the symptoms and changes characteristic of clinical "hyperthyroidism" are difficult to induce in animals by the administration of thyroid. In the two animals tried, powdered fresh sheep thyroid (Armour) was administered with food until a loss of body weight was demonstrated and diarrhea occurred. The salicyl excretion tests were then carried out.

Quinine was administered with the idea of inhibiting general metabolism thereby inducing an increase in excretion of salicylate. Only one animal (dog 18) was tried, the result obtained being the contrary of that anticipated.

In one animal (dog 18) a severe nephritis was induced by the administration of salicylate. The lesion was confirmed microscopically, and renal function as indicated by the marked accumulation of urea-nitrogen of blood and lessened urine output was markedly decreased. It was found that only 26.6 per cent of the total salicylate administered was excreted. This marked destruction is believed to be in line with that observed in human individuals with nephritis.

Summarizing the results with animals presented in table 6, it may be said that with the exception of dog 18, suffering with nephritis, increased destruction of salicylate was not demonstrated in abnormal states of metabolism attempted by the administration of quinine and thyroid, and in degenerative lesions of the liver by phosphorus. All of the results obtained are within the normal range of excretion studied in nine animals, a part of which served as controls before treatment with thyroid, quinine and phosphorus. Owing to the difficulties and uncertainties accompanying experiments of this type further sacrifice of animals was considered inadvisable.

It is interesting to note that the total excretion of salicylate in the eight untreated dogs and one cat that were studied as normals is individually and collectively much less than that in normal human individuals. This is to be interpreted as a greater capacity for destruction of salicylate.

TABLE 6

Excretion of salicyl by animals under different conditions

NUMBER AND ANIMAL	WEIGHT	TREATMENT	QUANTITY SALICYL* ADMINISTERED	SALICYL EXCRETED	REMARKS AND METHOD OF ADMINISTRATION
Normal (controls)					
	kgm.		*grams*	*per cent*	
Dog 19	5.8	None	1.24	55.43	Kidney showed some cloudy swelling; hypodermic
Dog 20	8.5	None	1.53	52.31	Gastric
Cat 21	4.0	None	0.77	58.2	Gastric
Dog 22	12.1	None	1.8	58.4	Gastric
Dog 23	8.3	None	0.9	71.4	Gastric
Dog 24	9.5	None	1.35	53.72	Gastric
Dog 25	5.6	None	0.9	56.6	Intramuscular
Dog 26	8.1	None	1.35	63.0	Intramuscular
Dog 28	19.4	None	3.15	55.84	Intramuscular
Median................................				56.6	
Hepatic degeneration (phosphorus)					
Dog 25	5.6	Control	0.9	56.6	Intramuscular
	4.5	After 0.008 gram phosphorus in oil	0.9	48.2	Marked emaciation; died after excretion was completed. Liver markedly degenerated. Intramuscular
Dog 28	19.4	Control	3.15	55.8	Intramuscular
		After 0.02 gram phosphorus in oil	2.88	51.7	Marked emaciation; died after excretion was completed. Liver degenerated. Intramuscular
Thyroid administration					
Dog 20	8.5	Control	1.53	52.3	Gastric
		Control	1.08	24.7	Intramuscular
	7.1	21 grams powdered thyroid	0.94	58.6	Emaciated, diarrhea; intramuscular
	7.1		0.85	64.2	Condition improved; diarrhea stopped; intramuscular
	8.6		0.85	70.6	In good condition; gastric
Cat 21	4.0	Control	0.77	58.2	Gastric
	3.0	9 grams powdered thyroid	0.72	50.5	Emaciated; gastric

TABLE 6—*Continued*

NUMBER AND ANIMAL	WEIGHT	TREATMENT	QUANTITY SALICYL* ADMINISTERED	SALICYL EXCRETED	REMARKS AND METHOD OF ADMINISTRATION
			Quinine		
Dog 20	7.1	Control	0.85	60.0	Intraperitoneally
	8.5	24 grams quinine sulphate	0.90	50.0	Intraperitoneally
			Nephritis (salicyl)		
Dog 18	4.0	Nephritis	1.3	26.6	Subcutaneously

* Expressed as salicylic acid.

6. *Influence of methods of administration on the excretion of salicyl*

The following tabulation derived from the data in Table 6 indicates that the quantitative excretion of salicylate is not influenced by the method of administration:

Method of administration	*Per cent of salicyl excretion in the same and different animals*
Subcutaneous	52.3; 54.7; 55.4
Gastric	56.4; 70.6; 71.4; 58.4; 58.2; 50.5
Intraperitoneal	60
Intramuscular	55.8; 56.6; 48.2; 58.6

III. DISCUSSION

Taken as a whole the results of the experiments described definitely indicate that the salicyl group is destroyed by living organisms. The destruction is not associated with any special organs; but rather appears to be a part of the general function of metabolism. This is indicated by the fact that destruction occurs by low organisms like fungi and probably by excised organs, and it is considerably augmented in conditions known to exhibit increases in metabolism (katabolism) such as fevers and Basedow's disease. Also, when there is retention, as in nephritis, considerably more destruction takes place than normally. On the other hand, when the liver was injured, excretion of salicyl was within

the normal range which indicates that the liver plays no special part in the destruction. The destruction in animals appears to be greater than in man.

That the salicylate group undergoes destructive changes in the body is contrary to the older views of Nencki (6) and Mosso (7), although it is a familiar chemical fact that salicylate when heated with alkalies is rather readily changed to phenol. Certain investigators (8) have failed to confirm the large excretion of salicyl reported by Nencki and Mosso. The evidences brought forward in this and previous papers (1) by us would seem to leave no doubt concerning the destruction.

The fact that the salicyl group is destroyed by living organisms should be of some practical interest in the metabolism of phenols and other aromatic derivatives. It may help to appreciate why salicyl in low concentrations is not an effective antiseptic in the blood and tissues; the necessity of using large doses to secure therapeutic effects; its harmlessness when used in small concentrations as food preservative, reference here, of course, not being made to free salicylic acid and its local effects.

IV. CONCLUSIONS

1. Solutions of sodium salicylate gradually deteriorate on standing, the loss being greater with weaker solutions.

2. The destruction is due to some form of living matter such as fungi, since solutions containing a preservative (chloroform), and free from fungi, do not deteriorate.

3. Yeast destroys salicylate, but not nearly as much as the fungus which naturally grows in salicylate solutions.

4. Treatment of salicylate with hashed animal organs results in considerable loss of the drug, which in part, at least, is due to destruction of salicyl. There is no difference between the action of liver and other organs.

5. About 20 per cent of salicylate administered to normal human individuals is destroyed, since the loss can not be accounted for in sweat and feces or by retention.

6. The destruction in animals (dog and cat) is even greater, amounting to about one-half of the salicyl administered.

7. The destruction of salicylate is markedly increased (about 40 per cent) in febrile conditions of man, drug habitués (alcohol and morphin), nephritis of both man and dog, and in Basedow's disease.

8. The destruction does not appear to be the special function of a given organ such as the liver, since the excretion in certain diseases of the liver in man and in hepatic degeneration in animals was within the normal range.

9. The increased capacity for destruction, therefore, may be ascribed to the general increase in metabolism (katabolism) of febrile conditions and Basedow's disease; to retention with prolonged exposure to the destructive action of the tissues in nephritis.

REFERENCES

(1) Hanzlik, Scott and Thoburn: Journ. Pharm. and Exp. Therap., 1917, ix, 247.
(2) Thoburn and Hanzlik: Journ. Biol. Chem., 1915, xxiii, 163.
(3) Dixon: Manual of Pharmacology, 1915, 39, Arnold, London.
(4) Dubois: Arch. Int. Med., 1916, xvii, 915; J. Am. Med. Assoc., 1914, lxiii, 827.
(5) Carlson, Rooks and McKie: Am. Journ. Physiol., 1912, xxx, 129.
(6) Nencki: Arch. f. Exp. Path. Pharm., 1886, xx, 367; Arch. f. Anat. u. Physiol., 1870, p. 399.
(7) Mosso: Arch. f. Exp. Path. Pharm., 1889, xxvi, 267.
(8) Wiley: U. S. Dept. Agriculture, Bur., Chem. Bull., No. 84, Part ii, 1906.
Feser and Friedberger: Arch. f. wissench., u. prakt. Tierheilk, 1875, H. 2, 3, and 6; Berl. klin. Woch., 1875, xii, 321.

THE SALICYLATES

XII. THE EXCRETION OF SALICYL AFTER THE ADMINISTRATION OF METHYL SALICYLATE TO ANIMALS

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The excretion experiments previously reported (1) deal exclusively with sodium salicylate. It would be interesting to observe the fate of other salicyl derivatives. Methyl salicylate was chosen and the experiments were carried out in the same way as those with sodium salicylate. In the recovery of salicyl attempts were made to differentiate between the salicyl which was excreted as the sodium salt and the ester itself. In the recovery of the ester it was only necessary to distil the freshly voided urine directly. The distillate, which contained the ester, was then hydrolyzed with sodium hydroxide, acidified with phosphoric acid and distilled for salicyl in the usual way. The remaining urine, after removal of the ester, was acidified with phosphoric acid and distilled for salicyl as sodium salicylate. The amount of sodium salicyl was also determined by difference (subtracting of ester) from the total salicylate recovered after hydrolysis of a fresh sample of urine. The administration of the ester in the majority of animals was gastric, and in one animal (Dog 30) intramuscular. The dosage was equivalent to the therapeutic "toxic dose" of sodium salicylate. The data obtained are presented in the accompanying table.

The results indicate a very low (range 23 to 37 per cent, mean about 25.5 per cent) recovery of salicyl, indicating that salicyl in the form of methyl salicylate is either more completely de-

TABLE

Excretion of salicyl by animals after administration of methyl salicylate

NUMBER AND ANIMAL	WEIGHT	QUANTITY SALICYL* ADMINISTERED	SALICYL EXCRETED	REMARKS AND METHOD OF ADMINISTRATION†
	kgm.	*grams*	*per cent*	
Dog 29	8.2	1.72	25.9	Excreted 0.44 gram as sodium salicyl; 0.003 gram as methyl salicyl = 0.2 per cent; gastric.
Dog 30	7.0	1.4	25.6	Excreted 0.204 gram as methyl salicyl = 14.4 per cent; 0.158 gram as sodium salicyl; intramuscular.
Dog 30	7.0	1.4	37.0	Excreted 0.542 gram as sodium salicyl; 0.005 gram as methyl salicyl = 0.36 per cent gastric.
Cat 21	3.0	1.0	23.0	Gastric; urine has wintergreen odor.
Dog 27	14.0	2.93	29.8	Excreted: 0.875 gram as sodium salicyl; 0.016 gram as methyl salicyl or 0.52 per cent: gastric.

* Expressed as salicylic acid.

† The duration of salicyl excretion was 5, 3, 4, 6 and 4 days for cat 21 and dogs 27, 29, 30 (intramuscular) and 30 (gastric), respectively.

stroyed than sodium salicylate, or what appears more probable, because of its great lipoid solubility, it is retained and the excretion towards the last is so small and prolonged that it is impossible to detect the small quantities excreted. The urine, of course, in each case was collected until salicyl-free as far as could be determined by ether extraction, hydrolysis and color tests.

A considerable portion of the ester is excreted as such. It was found in three animals (dogs 27, 29 and 30) that 0.2 per cent to 0.52 per cent of the total quantity administered by stomach was excreted as the unchanged ester. There was no doubt that methyl salicylate as such was present in urine, since all of the urines possessed the odor of wintergeen no matter whether the ester was administered into the stomach or intramuscularly. The ester was repeatedly shown to be present by direct ether extraction. As would be expected, one animal (Dog 30), which was injected intramuscularly, showed the presence of more (14.4 per

cent) of the unchanged methyl salicylate in urine than the same animal when the same quantity was administrated into the stomach, indicating that a considerable portion of the ester can be hydrolyzed in the alimentary tract.

The small quantity of salicyl recovered after the administration of methyl salicylate is not due to the relatively small quantities administered, since higher recoveries were obtained with corresponding small quantities of sodium salicylate as reported in a previous paper. Also the uniformity of results obtained is against loss of the drug with feces. This was entirely excluded with sodium salicylate, and in the particular animals studied with methyl salicylate the animals passed practically no feces during the course of the experiments. For the present, the fate of the unexcreted methyl salicylate remains undetermined.

It is of considerable interest to know that the ester is excreted as such to considerable concentrations (0.2 per cent to 0.52 per cent) in the urine. How important this may be from the standpoint of analgesia, systemic and urinary antisepsis is not known. Methyl salicylate is known to possess greater toxicity and analgesic properties, than sodium salicylate and this may be connected with the presence of the ester itself in the systemic circulation and tissues.

Nencki (2) and Lesnik (3) considered the salicyl esters to be almost completely hydrolyzed in the intestine. However, this has been denied by Baas (4). Nencki and Lesnik worked principally with salol (phenyl salicylate) and the resorcin ester of salicylic acid. According to Baas, salol is the most easily decomposable of the salicyl esters and probably none is absorbed as such. Baas concluded from his work that both the methyl and ethyl salicylate are absorbed to a considerable extent unchanged, differing in this respect from salol. He also observed that the urine of dogs receiving methyl salicylate possessed the odor of wintergreen. Our experiments confirm the presence of unhydrolyzed ester in urine, the oily ester with characteristic odor having been repeatedly recovered from urine by ethereal extraction.

CONCLUSIONS

1. The excretion of salicyl by animals (dogs and a cat) after the administration of methyl salicylate is much less (25 per cent) than after sodium salicylate previously reported.

2. After gastric administration the free ester was found in urine in the concentrations of 0.2 per cent to 0.52 per cent, and 14.4 per cent after intramuscular injection.

3. This may be of importance in explaining the greater analgesic properties and toxicity possessed by methyl salicylate, and in urinary and systemic antisepsis.

REFERENCES

(1) Hanzlik and Wetzel: Journ. Pharm. Exp. Therap., 1919, xiii, 25.
(2) Nencki: Arch. f. exp. Path. Pharm., 1886, xx, 367; Arch. f. Anat. und Physiol., 1870, 399.
(3) Lesnik: Arch. f. exp. Path. Pharm., 1888, xxiv, 171.
(4) Baas: Zeit. f. physiol. Chem., 1890, xiv, 416.

THE EFFECTS OF CHLORINE UPON ISOLATED BRONCHI AND PULMONARY VESSELS

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The behavior of the smooth muscle of the lung in the presence of chlorine may be related to the production of pulmonary edema, an influence being exerted by changes in the tone of either the vascular or the bronchial musculature.

A diminution in caliber of the pulmonary veins will curtail the blood flow, tending to produce congestion and edema. Vascular relaxation could contribute to the production of edema if associated with increased permeability of the vessel walls.

Bronchoconstriction, if produced, may favor the occurrence of edema by increasing the negative pressure in the alveoli, each deep inspiration exerting a "cupping" effect upon the capillaries, as suggested by Gates and Auer.[2]

The behavior of excised rings of bronchi and pulmonary vessels in the presence of chlorine was submitted to the following investigation.

METHOD

Tissues from fresh pig's lungs were placed on ice immediately after slaughter and used within three hours. In two cases calves' bronchi were substituted (see table 3).

Rings were cut from arteries, veins, or bronchi, as small as could be conveniently handled. They were dissected with clean instruments, other contact being avoided as much as possible.

[1] Published with the permission of the Director of the Chemical Warfare Service.

[2] Gates, F. L., and Auer, J., J. Pharm. & Exp. Therap., 1917, ix, 361.

The surrounding tissue (including cartilage in the case of the bronchi) was carefully removed, the rings being kept moist with Locke's solution.

TABLE 1

Chlorine on pulmonary veins

EXPERIMENT NUMBER	CHLORINE PER LITER	DIAMETER OF RING	RELAXATION	CONTRACTION
	mgm.	*cm.*		
8-2	32	0.5	+	0
7-3	74	0.75	+	0
7-1	111	0.75	+	0
7-2	148	0.75	+	0
6-3	205	0.7	+	0
6-2	205	0.7	+	0
8-3	322	0.5	+	+
8-1	322	0.5	+	+
6-1	410	0.7	?	++
3-2	620		?	+++

TABLE 2

Chlorine on pulmonary arteries

EXPERIMENT NUMBER	CHLORINE PER LITER	DIAMETER OF RING	RELAXATION	CONTRACTION
	mgm.	*cm.*		
9-5	28	0.75	+	0
10-2	63	0.75	+	0
10-1	126	0.75	+	0
5-2	129		+	0
9-3	138	0.75	+	0
10-3	252	0.75	+	0
5-1	259		?	+
9-1	276	0.75	+	+
9-4	414	0.75	0	+
4-1	536		0	++
9-2	552	0.75	0	+
3-1	620		0	+++

A prepared ring of tissue was suspended in a glass cylinder containing Locke's (dextrose free) solution. Below, it was attached by a silk thread to the drawn out end of a glass tube through which oxygen was supplied. Above, a platinum hook and thread served to connect the tissue to a writing lever by

means of which its activity was recorded upon a slowly moving drum. The lever magnification was 21:1 unless otherwise noted. The counterweight employed was slightly more than sufficient to keep the thread taut. The glass cylinder was equipped with a siphon for changing the contents. By means of a water bath the whole was kept at a constant temperature of 37.5°C.

TABLE 3

Chlorine on bronchi

EXPERIMENT NUMBER	CHLORINE PER LITER	DIAMETER OF RING	RELAXATION	CONTRACTION
	mgm.	*cm.*		
17–2	55	2.75	0	0
12–1	160	1.5	0	+
12–3	160	1.25	+	?
15–3	170	2.5	+	0
13–1	186	1.5	0	+
16–3	197	2.75	0	+
16–2	312	2.0	+	+
15–2	340	2.5	?	+
1–2	380		0	+
14–1	404	1.5	+	+
17–1	495	2.5	0	+
16–1	624	2.5	0	+++
15–1	680	2.5	+	+++
2–1	936	*	0	++
2–2	1248	*	0	+++

* Calf bronchus.

Locke's solution was employed, oxygen being added at a uniform rate. The tissue to be tested was suspended in this solution and its action noted until the muscular tone had maintained a constant level for a number of minutes. Chlorinated Locke's solution of the same temperature was then added. The total fluid present after this addition was 50 cc.

Locke's solution was freshly chlorinated each day, and protected from the action of light when not in use. The analyses were made by Mr. F. A. Taylor. The figures given represent in each case an average of three analyses.

RESULTS

The results may be summarized best in the accompanying tables. The degree of each effect is indicated roughly by the number of plus signs. A zero signifies that no effect was exhibited. Typical tracings from each type of smooth muscle employed are also presented.

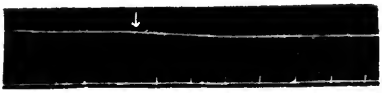

Fig. 1

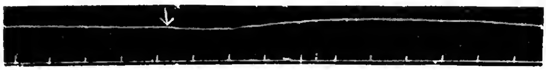

Fig. 2

Fig. 3

Figs. 1–3. Chlorine on Pulmonary Veins

Upstroke = contraction (lever magnification 21: 1). Time = 1 minute intervals.

Fig. 1. (Curve 6–3) Pulmonary vein, pig. 205 mgm. Cl_2 per liter Locke's solution, Cl_2 added at arrow.

Fig. 2. (Curve 8–1) Pulmonary vein, pig. 322 mgm. Cl_2 per liter Locke's solution; Cl_2 added at arrow.

Fig. 3. (Curve 3–2) Pulmonary vein, pig. 620 mgm. Cl_2 per liter Locke's solution, Cl_2 added at arrow No. 1; changed back to Locke solution at arrow No. 2.

It is readily seen that the presence of even small concentrations of chlorine leads to a slight relaxation of pulmonary vessels (figs. 1 and 4). When a concentration approximating 250 milligrams of chlorine to one liter of Locke's solution is attained the same response occurs but is soon succeeded by a slow and prolonged contraction of the muscle (figs. 2 and 5).

High concentrations, e.g., 620 mgm. per liter, produce no relaxation but a rapid and extensive contraction (figs. 3 and 6). These vascular rings will however relax again if the solution be replaced by one which is chlorine-free.

The bronchial musculature (figs. 7–10) yields similar responses to chlorine but the relaxation occurs with less constancy. It

Fig. 4

Fig. 5

Fig. 6

Figs. 4–6. Chlorine on Pulmonary Arteries

Upstroke = contraction (lever magnification 21: 1). Time = 1 minute intervals.

Fig. 4. Curve 9-3. Pulmonary artery, pig. Diameter: 7.5 mm. 138 mgm. Cl_2 per liter Locke's solution, added at arrow.

Fig. 5. Curve 5-1. Pulmonary artery, pig. 259 mgm. Cl_2 per liter Locke's solution, added at arrow.

Fig. 6. Curve 3-1. Pulmonary artery, pig. 620 mgm. Cl_2 per liter Locke's solution, added at first arrow; Cl_2 washed out at second arrow.

was marked however in one case where the concentration was as high as 680 mgm.—being succeeded by the usual constriction produced by such amounts (fig. 9). Another difference between vessels and bronchi lies in the fact that for the latter the minimal constricting concentration is much lower than for the former, less than 200 mgm. per liter sufficing to contract the bronchial rings.

Fig. 7

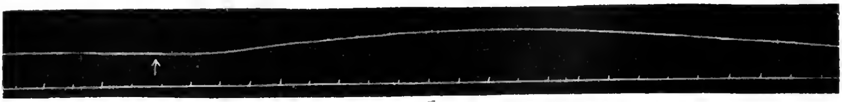

Fig. 8

Fig. 9

Fig. 10

Figs. 7–10. Chlorine on Bronchi

Upstroke = contraction (lever magnification in figures 7–9, 21:1.) Time = 1 minute intervals, except figure 10.

Fig. 7. (Curve 15–3). Bronchial muscle, pig. (Diameter: 2.5 cm.) 170 mgm. Cl_2 per liter Locke's solution, added at arrow.

Fig. 8 (Curve 13–1.) Bronchial muscle, pig. (Diameter 1.5 cm.) 186 mgm. Cl_2 per liter Locke's solution; added at arrow.

Fig. 9. (Curve 15–1.) Bronchial muscle, pig. (Diameter 2.5 cm.) 680 mgm. Cl_2 per liter Locke's solution, added at arrow.

Fig. 10. (Curve 2–2.) Slow speed drum, tracing covers $2\frac{3}{4}$ hours (lever magnification 10:1). Bronchial muscle, calf. 1248 mgm. Cl_2 per liter Locke's solution, added at arrow.

CONCLUSIONS

Isolated sections from all three of the pulmonary systems, venous, arterial and bronchial, relax slightly in Locke's solution, under the influence of low chlorine concentrations, but contract vigorously in the presence of greater amounts. Medium concentrations (300 mgm. chlorine per liter of Locke's solution) produce relaxation followed by constriction.

The broncho-constriction by 600 mgm. per liter is probably sufficient to augment an edematous condition by means of the increased negative pressure in the alveoli with each inspiration.

A constriction of the pulmonary veins as extensive as such concentrations produce should, occurring in vivo, prove distinctly favorable to the occurrence of pulmonary congestion and edema.

Measures tending to relax the bronchi and the pulmonary vessels may have a favorable influence upon lungs poisoned by chlorine.

DRUGS AFTER CHLORINE GASSING

I. THE INFLUENCE OF MORPHINE UPON THE FATALITY OF CHLORINE POISONING

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The following experiments were undertaken to determine the effect of morphine injections upon the fatality percentage in dogs gassed with chlorine.

Forty-six dogs were gassed with a constant chlorine concentration (approximately 0.08 per cent by volume); of these thirty-five were treated with one or more subcutaneous injections of morphine sulfate, the remaining eleven serving as controls.

The term "recovery" ("R") as used in this report indicates a survival of at least seventy-two hours, a period which exceeds the maximum duration of the acute stage of chlorine poisoning.

FATE OF GASSED DOGS NOT TREATED WITH MORPHINE

In the following series of seven dogs we obtained no recoveries; the average survival period was only 11.3 hours:

Series 1. Gassed dogs; untreated

DOG		CHLORINE	SURVIVED
Number	Species		
		p.p.m.	*hours*
A41	Mongrel	848	25
A42	Bull	858	14–22
A43	Collie	835	5
A44	Collie	866	4
A45	Collie	746	3
A47	Terrier	851	12–20
A52	Collie	791	8
Average			11.3

[1] Published with the permission of the Director of the Chemical Warfare Service.

Of four gassed dogs subjected to bleeding during the first day one recovered. The average survival period of the other three was 19.8 hours:

Series 2. Gassed dogs; 15 cc. Bleeding at two to three hour intervals

DOG		CHLORINE	SURVIVED
Number	Species		
		p.p.m.	*hours*
D12	Collie	907	14–24
D14	Collie	866	R
D16	Collie	787	26–28
D25	Hound	834	11–16
Average			19.8

There was therefore but one recovery among eleven dogs gassed with the standard "lethal" concentration and not treated with morphine. Under the conditions which obtained in this division one dog in seven, or 15 per cent, usually survived such a concentration of chlorine.

FATE OF GASSED DOGS TREATED WITH MORPHINE

Of six dogs treated with subcutaneous injections of 10 mgm. per kilo morphine sulfate repeated every ten to twelve hours none recovered. The average survival period was 15.4 hours:

Series 3. Morphine in gassed dogs (10 mgm. per kilo)

DOG		CHLORINE	SURVIVED	REMARKS
Number	Species			
		p.p.m.	*hours*	
A72	Collie	882	28–33	3 injections, 10 hour intervals
A73	Hound	849	$6\frac{1}{2}$	
A75	Terrier	894	25	3 injections, 11 hour intervals
A78	Terrier	828	$7\frac{1}{2}$–9	
A79	Bull	860	12–22	2 injections, 12 hour intervals
A80	Mongrel	834	3–7	
Average			15.4	

Ten dogs similarly treated with morphine were bled at two to three hour intervals throughout the first day. One of these recovered from the acute stage but died after four and one-half days. The average survival period of the others was 21.2 hours:

Series 4. Morphine in gassed dogs (10 mgm. per kilo). 15 cc. Bleeding at two to three hour intervals*

DOG		CHLORINE	SURVIVED	REMARKS
Number	Species			
		p.p.m.	*hours*	
D4	Terrier	911	32	4 injections
D6	Irish terrier	846	R	9 injections (died in $4\frac{1}{2}$ days)
D7	Mongrel	869	12–24	2 injections
D8	Coach	811	30	2 injections
D9	Bull	857	12–24	2 injections
D10	Mongrel	867	12–24	2 injections
D13	Collie	886	$30\frac{1}{2}$	3 injections
D15	Collie	825	$6\frac{1}{2}$	
D17	Shepherd	851	26–28	3 injections
D24	Hound	834	11	
Average			21.2	

* The alkali reserve of the blood was studied in these dogs, no lasting effect of morphine being demonstrated. Hjort, A. M. & Taylor, F. A., Journ. Pharm. & Exp. Ther., 1919.

Of three dogs similarly treated with morphine and exposed to an external temperature of 33°C. the average survival period was only 3.5 hours:

Series 5. Morphine in gassed dogs (10 mgm. per kilo), in warm box at 33°C.

DOG		CHLORINE	SURVIVED
Number	Species		
		p.p.m.	*hours*
A74	Mongrel	891	3
A76	Spaniel	880	$3\frac{1}{2}$
A77	Terrier	888	$3\frac{1}{2}$–$4\frac{1}{2}$
Average			3.5

Effects of smaller doses of morphine

Of eleven dogs treated each with a single subcutaneous injection of 5 mgm. per kilo morphine sulfate one recovered. For the remaining ten animals the average survival period was 12.6 hours:

Series 6. Morphine in gassed dogs (5 mgm per kilo)

DOG		CHLORINE	SURVIVED	REMARKS
Number	Species			
		p.p.m.	*hours*	
A105	Terrier	858	10–20	
A108	Terrier	891	30–40	
A109	Hound	820	3–5	
A110	Spaniel	901	6–15	
A111	Terrier	893	$6\frac{1}{2}$	
A112	Pug	893	1–3	
A113	Terrier	869	$10\frac{1}{2}$–20	
A114	Bull	874	R	
A117	Pointer	878	$11\frac{1}{2}$–$20\frac{1}{2}$	Kept at 28°C.
A119	Mongrel	847	$9\frac{1}{2}$–19	
A121	Pointer	869	12–23	
Average			12.6	

Of five gassed dogs treated each with a single subcutaneous injection of 3 mgm. per kilo morphine sulfate one recovered but died after five days. The others survived an average of 15.4 hours:

Series 7. Morphine in gassed dogs (3 mgm. per kilo)

DOG		CHLORINE	SURVIVED	REMARKS
Number	Species			
		p.p.m.	*hours*	
A134	Terrier	896	R	Died in 5 days
A135	Terrier	896	11–20	
A136	Setter	851	$31\frac{1}{2}$–$41\frac{1}{2}$	
A137	Collie	832	3–8	
A138	Shepherd	896	2–7	
Average			15.4	

SUMMARY OF FATALITIES

The above results may be condensed into the following table:

Dogs gassed with approximately 0.08 per cent chlorine

SERIES	TREATMENT	NUMBER OF DOGS	RECOVERED	AVERAGE HOURS SURVIVAL IN ACUTELY FATAL CASES
1	None	7	0	11.3
2	Bleeding	4	1	19.8
3	Morphine 10 mgm. per kilo	6	0	15.4
4	Morphine 10 mgm. per kilo + bleeding	10	1	21.2
5	Morphine 10 mgm. per kilo + heat	3	0	3.5
6	Morphine 5 mgm. per kilo	11	1	12.6
7	Morphine 3 mgm. per kilo	5	1	15.4

The series may be grouped as follows:

SERIES	TREATMENT	NUMBER OF CASES	PER CENT RECOVERED
1–2	No morphine	11	9.1
3–5	Morphine, 10 mgm. per kilo	19	5.3
6–7	Morphine, 3 to 5 mgm. per kilo	16	12.5

CONCLUSIONS

1. Subcutaneous doses of morphine as large as 10 mgm. per kilo (given twice daily) exert, if anything, an unfavorable effect upon chlorine gassed dogs.

2. Smaller doses (3 to 5 mgm. per kilo) do not appear to influence significantly the fatality percentage.

3. It would appear that morphine may safely be used in gassed individuals, for its analgesic effect, if the size and frequency of the doses are limited.

DRUGS AFTER CHLORINE GASSING

II. OBSERVATIONS UPON THE TREATMENT OF GASSED DOGS WITH CIRCULATORY STIMULANTS

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The following data relate to attempts to control the effects of chlorine gassing in dogs by means of circulatory stimulants. The effects of epinephrin (commercial adrenalin chloride) and of ouabain (crystalline g.-strophanthin) were investigated.

After gassing with a concentration of chlorine of 800 to 900 parts per million each dog received immediately a 5 mgm. per kilo subcutaneous injection of morphine sulphate. This procedure while quieting to the animal has been shown, in a previous paper,[2] to exert no demonstrable effect upon the result of chlorine gassing.

The term "recovery" ("R") as used in this report indicates a survival of at least seventy-two hours.

EPINEPHRIN

As soon as the animal became quieted by morphine, epinephrin was administered in 1: 1,000 solution, or weaker, by means of two or three extremely slow intravenous injections, separated by intervals of from three to five minutes.

Each injection resulted in characteristic manifestations of epinephrin action, as judged from marked temporary augmentation of pulse volume as well as of rate and depth of respiration.

[1] Published with the permission of the Director of the Chemical Warfare Service.

[2] Barbour, H. G., Hjort, A. M., Taylor, F. A. Paper I.

In the series of twenty experiments summarized in table 1, although five recoveries were obtained, it will be seen that these occurred all within the first nine tests. After experiment A91 not a single animal could be saved. No reason for this sudden change in the results could be found. In the first ten experiments the recovery percentage was fifty; in the last ten it was zero. Four of the five animals which recovered were in good health a month later.

TABLE 1

Treatment of chlorine-gassed dogs with epinephrin. "R" = recovered

Dog			Chlorine	Epinephrin (intravenous)		Survived	Remarks
Number	Species	Kilos					
			p.p.m.	*mgm.*	*mgm. per kilo*	*hours*	
A82	Mongrel	17.0	831	0.45	0.03	13–22	
A83	Collie	10.5	809	0.45	0.04	R	
A84	Mongrel	15.5	833	1.0	0.06	R	
A85	Spaniel	13.5	833	2.0	0.15	R	Kept at 33°C. 2 days
A87	Terrier	9.0	890	2.0	0.22	11–20½	
A88	Terrier	13.0	831	3.0	0.23	6–11	
A89	Terrier	8.5	879	2.0	0.24	9–18	
A90	Collie	13.5	866	3.0	0.22	R	Died after 5 days
A91	Terrier	13.5	899	3.0	0.22	R	
A92	Pointer	16.0	820	3.2	0.20	6½–15½	
A94	Terrier	14.0	858	3.0	0.21	2½–4	
A96	Terrier	12.0	858	3.0	0.25	6–11	Kept at 33°C.
A98	Spaniel	12.5	853	2.5	0.20	19–21½	
A99	Irish terrier	11.0	861	2.0	0.18	2–8	
A100	Mastiff	25.0	821	5.0	0.20	1½–7	
A101	Mongrel	13.0	858	2.5	0.19	30–40	
A102	Mongrel	13.0	805	2.5	0.19	5–15	
A104	Spaniel	16.0	883	3.2	0.20	6–8	3.2 mgm. repeated after 3 hours
A106	Terrier	13.5	872	3.0	0.22	3½–5	3.0 mgm. repeated after 3 hours
A107	Collie	11.0	871	3.0	0.27	4–6	Kept at 33°C.
Average						11.3	

In an additional experiment, A97, the morphine was omitted, 0.23 mgm. per kilo epinephrin being given. Death followed within twenty-four hours.

Two further experiments (A93 and A95) were made with tyramine hydrochloride, a stimulant similar to epinephrin. Subcutaneous doses of 3.3 and 3.8 mgm. per kilo respectively were employed. In the latter case no morphine was used. Both animals died within 21 hours.

OUABAIN

In testing this drug the procedure was followed of delaying administration until the pulse began to show weakness and increased frequency. Preliminary tests showed that as much as 0.05 mgm. per kilo could be given to normal dogs without untoward effects. This amount given by intramuscular injection produces in gassed dogs a temporary recovery of the failing circulation. Table 2 shows however that the chances of fatality cannot be said to be diminished. Of fourteen dogs, each treated with a single 0.05 mgm. per kilo injection, only two recovered

TABLE 2

Treatment of chlorine-gassed dogs with ouabain

DOG			CHLORINE	OUABAIN (INTRAMUSCULAR	INTERVAL BETWEEN GASSING AND INJECTION		SURVIVED	REMARKS
Number	Species	Kilos						
			p.p.m.	*mgm. per kilo*	*hours*	*minutes*	*hours*	
A116	Collie	15	824	0.05	6	0	60–69	
A118	Setter	19	864	0.05	2	10	R	Survived 16 days
A120	Terrier	11	875	0.05	2	0	$4\frac{1}{2}$	
A122	Terrier	8	890	0.05	1	30	$3\frac{1}{2}$	
A123	Collie	16	853	0.05	2	40	$6\frac{1}{2}$	
A124	Mongrel	15	893	0.05	1	40	$2\frac{1}{2}$–4	
A125	Mongrel	14	869	0.05	1	10	$3\frac{1}{2}$–5	
A126	Collie	25	853	0.05	1	0	$4\frac{1}{2}$–11	
A128	Collie	25	854	0.05	2	0	$7\frac{1}{2}$	
A129	Collie	21	897	0.05	1	40	$19\frac{1}{2}$	
A130	Mongrel	11	888	0.05	1	20	6–16	
A131	Mongrel	15	874	0.05	0	40	R	Survived 7 days
A59	Collie	11	830	0.05	0	50	2–4	
A60	Airdale	12	859	0.05	0	20	4–8	
A115	Collie	17	874	0.1	2	0	3–4	
A127	Collie	19	874	0.05	0	35	$10\frac{1}{2}$–20	Injection repeated after 10 hours
A132	Setter	15	887	0.03	2	0	$13\frac{1}{2}$–$22\frac{1}{2}$	Injection repeated after 3 hours
A133	Hound	17	842	0.03	1	30	5	
Average							11.9	

(14.3 per cent). The last four cases in this table illustrate unsuccessful variations of this treatment.

It may be said of both epinephrin and ouabain that no essential differences between treated and untreated gassed dogs were noted. The signs of pulmonary disturbance were of similar intensity. The animals which recovered did not improve with greater rapidity than those which survive chlorine gassing untreated.

CONCLUSION

It is improbable that either epinephrin or ouabain can be made to exert a favorable influence upon chlorine poisoned dogs.

The author desires to thank Messrs. E. Fisher and F. A. Taylor for valuable assistance.

THE EFFECTS OF CHLORINE UPON THE BODY TEMPERATURE

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The following work is reported to show the effects upon the body temperature of chlorine gas in low and high concentrations. The experiments were all made upon healthy dogs gassed by the standard method of the Chemical Warfare Service.[2]

Three orders of concentration were employed: (1) 24 to 30 parts of chlorine by volume per million of air; (2) 180 to 200 parts per million; (3) 800 to 900 parts per million.

GENERAL OBSERVATIONS

A general picture of the action of these three concentrations will first be presented.

Gassing procedure without chlorine. One-half hour exposures of two normal dogs to a breeze of 250 liters per minute of chlorine-free air under the usual experimental conditions of gassing failed to alter the body temperature. Pulse, respiration and general behavior were unaffected.

Effects of 24 to 30 parts of chlorine per million. This concentration proved irritant but the four dogs exposed to it returned to an apparently normal condition immediately after removal from the chamber. During the gassing lacrimation and profuse salivation usually occurred as well as mild retching and vomiting. The effects upon pulse and respiratory rates were variable.

[1] Published with the permission of the Director of the Chemical Warfare Service.

[2] The method, which was developed in this Section, has been described by Prof. F. P. Underhill: The Lethal War Gases; Yale University Press, 1919.

Effects of 180 to 200 parts of chlorine per million. Three dogs were gassed with this concentration. The irritant effects described above were followed by general muscular depression and dyspnoea before the half hour gassing was terminated. A decided fall in pulse rate was observed. The changes of the respiratory rate were inconstant; no evidence of bronchitis or edema could be obtained. The animals became apparently normal again after a few hours.

Effects of 800 to 900 parts of chlorine per million. This concentration is fatal in at least 85 per cent of dogs which are exposed to it for half an hour. The clinical picture varies in individual cases but presents in general an intensification of the symptoms described above. During the gassing are frequently seen lacrimation, salivation, retching, vomiting, defecation, urination, dyspnoea, and profound muscular depression.

In a series of twenty-two dogs gassed with this concentration characteristic pulse changes occurred in every animal but one. These consisted in a marked slowing of the rate with increased amplitude succeeded within a few hours by the weak rapid pulse of circulatory collapse. The respiratory rate was usually much increased by the gassing, but in six cases marked especially by muscular depression and dyspnoea it was retarded.

In the seven dogs of this series which remained untreated bronchitis, edema and collapse developed rapidly, death ensuing in from three to twenty-five hours.

BODY TEMPERATURE

When one follows the rectal temperature in these three classes of cases a typical curve is obtained for each order of concentration of chlorine.

The lowest (irritant) concentrations (24 to 30 parts per million) produced during the gassing a rise of temperature averaging 0.8°C. in four dogs. The duration of the hyperthermia varied from three to twenty-four hours.

The medium (irritant-depressive) concentrations (180 to 200 parts per million) resulted in three dogs in an average temperature decrease of 0.7°C. during the gassing. Return to normal began

promptly thereafter and the average duration of the hypothermia was six hours.

The highest (lethal) concentrations (800 to 900 parts per million) produced likewise a temperature fall. The average change during gassing in 20 cases was −1.0°C., but from this there was no recovery in untreated cases. The temperature continued to fall at the same rate for about two hours after which it declined more gradually until death ensued.

These three types of temperature variation are illustrated in figure 1, in which each curve is a composite from three or more dogs gassed at the same concentration.

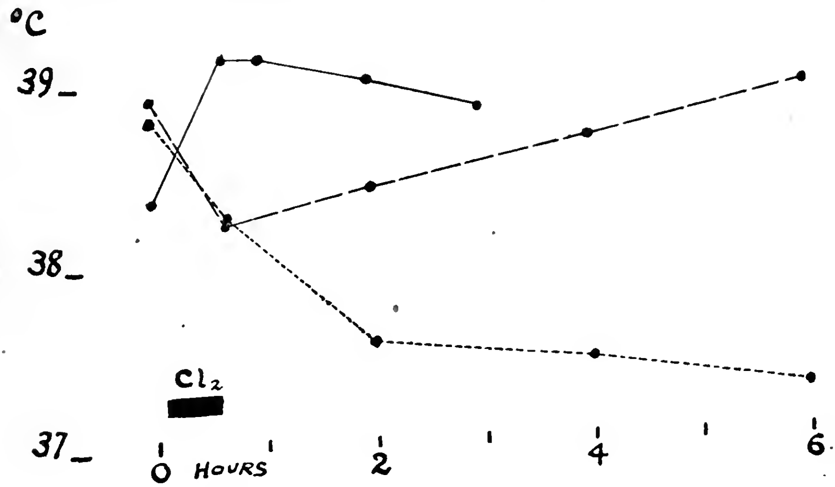

Fig. 1. Body Temperature of Dogs Exposed to Certain Concentrations of Chlorine

Unbroken line: 24 to 30 parts per million (composite of 4 curves); Dash line: 180 to 200 parts per million (composite of 3 curves); Dotted line: 800 to 900 parts per million (composite of 3 curves). Gassing period ("Cl_2) from fifth to thirty-fifth minute.

ARE THE TEMPERATURE CHANGES RELATED TO FREQUENCY OF PULSE OR RESPIRATION?

Before attempting an interpretation of the above described changes in body temperature the question of their relationship to variations in respiratory and pulse rates may first be disposed

of. The following table sums up the immediate effects upon temperature, pulse and respiration in the three series of cases:

This table shows at once the lack of any relation between respiratory rate and body temperature after chlorine gassing.

On the other hand is seen the constant pulse fall in the two series in which enough chlorine was given to depress the temperature. It was found possible, however, to produce with chlorine an equally profound fall in temperature without affecting the

TABLE 1

Temperature, respiration and pulse changes during chlorine gassing

CHLORINE	NUMBER OF CASES	AVERAGE CHANGE		
		Temperature	Pulse	Respiration
p.p.m.		°C.		
24–30	4	+0.8		
	1		0	
	1		−16	
	2		+20	
	1			−4
	1			0
	2			+8
180–200	3	−0.7	−41	
	1			+21
	2			−8
800–900	20	−1.0	−37	
	13			+10
	6			−6

pulse rate. This was done by gassing dogs in which central vagus influences had been eliminated.

In one case this was done by the subcutaneous administration of atropine sulphate (1 mgm. per kilo) fifteen minutes prior to gassing. In two other cases both vagi had been sectioned under local anesthesia on the previous day. The three dogs received 800 to 900 parts per million of chlorine and exhibited an average temperature decrease of 1.2°C. But the pulse rate remained unchanged, (except in one of the vagotomized animals in which it fell only from 176 to 168 beats per minute).

The pulse fall produced by lethal concentrations of the gas stands therefore in no causal relation to the temperature decrease.[3] We may then answer in the negative the question as to a relationship between respiratory or pulse rates on the one hand and body temperature upon the other in chlorine poisoning.

INTERPRETATION OF TEMPERATURE EFFECTS

Chlorine hyperthermia. Until further studies are made of the temperature change produced by low concentrations (24 to 30 parts per million) of chlorine we may class this tentatively as an "irritation hyperthermia." Obviously there is induced a derangement of the heat regulating mechanism by which the excessive heat produced by restlessness, abnormal secretory stimulation, etc., is prevented from being eliminated.

Chlorine hypothermia. The higher concentrations of chlorine produce a more serious derangement; the animal exposed to 180 or more parts of chlorine per million of air is unable to retain a normal amount of heat in the body. When the concentration was but 200 parts per million the heat-regulating function was restored upon removal from the gassing chamber. On the other hand when lethal concentrations were employed it was never regained (see fig. 1).

Before the conclusion of gassing with either of these concentrations one frequently observes general muscular depression; the action of a central anesthetic is suggested. On the other hand the temperature drop is equally profound in the absence of this depression. The chief factor was therefore presumed to be increased radiation of heat, and to establish this point two further series of experiments may be described: (1) the gassing of long-haired dogs; (2) the exposure of gassed dogs to high external temperatures.

(1) *Effects of gassing on long-haired dogs.* Three collie dogs gassed with approximately 800 parts of chlorine per million all yielded an

[3] Incidentally it is proven that the pulse slowing is due to a heightened tone of the vagus center. Cf. the observations upon anesthetized animals made by Schulz, W. H. Jour. Pharm. & Exp. Therap., 1918, xi, 179.

unusual result, namely a rise in temperature. The average increase for the three found immediately after gassing amounted to over 0.8°C. After a further hour the increase amounted to 1.3°C. These facts are illustrated in the upper curve of figure 2. Collapse set in during the third hour, an early death occurring in each case.

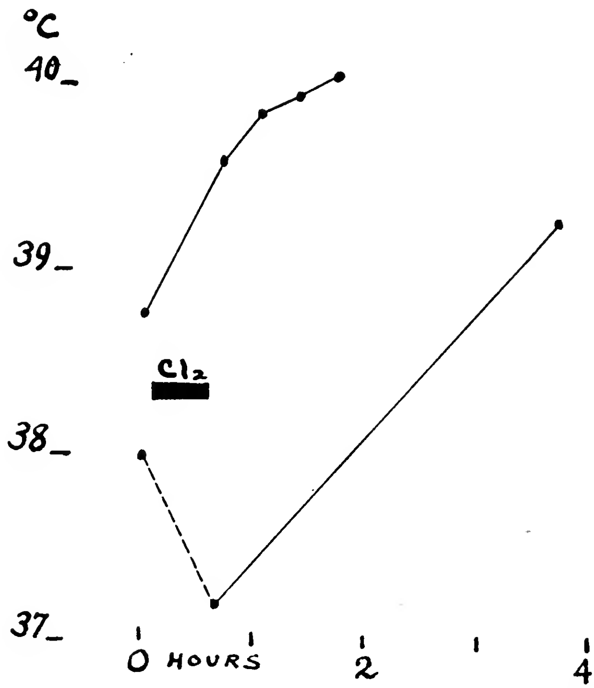

FIG. 2. EFFECTS OF LIMITED RADIATION OF HEAT IN DOGS GASSED WITH LETHAL CONCENTRATIONS OF CHLORINE (COMPARE WITH LOWEST CURVE, FIGURE 1)

Upper curve: Body temperature of long haired dogs gassed with 800 to 900 parts of chlorine per million (composite of 3 curves). Lower curve: Body temperature of a short haired dog kept in an environment of 38°C. after exposure at room temperature to the same concentration of chlorine. Gassing period ("Cl_2") from fifth to thirty-fifth minute.

Here then is seen another picture of an irritation *hyperthermia*, more severe than is produced by the lower concentrations. The same processes are doubtless set up when other than long-haired

dogs are gassed with high concentrations but are overcompensated in the latter case by increased radiation. Long hair excludes this factor.

(2) *Effects of exposure of gassed dogs to high external temperatures.* In a number of experiments short-haired dogs were, for the three hours immediately following gassing, exposed to an abnormally high external temperature. This was done by placing the animal in an insulated box, somewhat smaller than the gassing chamber, through which hot air dried by calcium chloride was drawn. When the temperature at the outflow of this warm box was 40°C. three different ungassed dogs were able to maintain a normal body temperature for three hours. Gassed animals on the other hand showed a steadily rising curve when the box was kept at 35°C. or any higher temperature. The lower curve (fig. 2) illustrates the temperature of a dog which lost 0.8°C. by gassing but three hours later was found to be 1.3°C. above the initial temperature. This was due to his being kept for the latter period in the warm box, the outflow temperature of which was maintained at 38°C.

From the last series of experiments it may be seen that the high chlorine concentrations render dogs unable to regulate against moderately high external temperatures (35 to 40°C.) as well as against moderately low ones (ordinary room temperatures) such as are readily tolerated by normal animals. This poikilothermic tendency is seen also under anesthetics. Morphine, for example, renders an animal prone to assume the temperature of its environment; the same is true of decerebration; but more specific involvement of the temperature-regulating mechanism is seen in the gassed dog, inasmuch as loss of consciousness and of motor control are not constant accompaniments of the hypothermia.

The nature of the derangement of the temperature-regulating mechanism resulting in hyperthermia and hypothermia respectively, after low and high concentrations of chlorine, is not yet clear. It is conceivable that the peripheral nerve irritation may be of sufficient intensity to exert an inhibitory effect upon the

temperature centers.[4] Underhill's[5] demonstration of a parallelism between blood concentration and body temperature after exposure to members of the chlorine series of gases suggests that excess of fluid in the circulation is detrimental to heat regulation. It is likely that in the plethoric condition too large a proportion of blood is kept at the surface of the body and thus exposed to environmental temperatures. To suggest the numerous interrelations of these two hypotheses would lead us too far into the field of speculation.

TEMPERATURE AND TREATMENT OF GASSED INDIVIDUALS

On the practical side, the facts brought out in this work emphasize the importance of assisting the body to maintain a normal temperature after gassing, which so obviously cripples the heat-regulating mechanism. Most authorities emphasize sufficiently the importance of keeping the temperature high enough but it seems pertinent to call attention to the ease with which the body, as in the warm box experiments, may become overheated.

Some attempts were made in connection with this work to establish an optimum environmental temperature but none was found at which the percentage of fatalities from 800 to 900 parts per million of chlorine was lowered significantly. However nine dogs gassed with this lethal concentration were kept at 33°C. for the forty-eight hours immediately following gassing unless death ensued earlier. Out of these there were two animals, both spaniels, which recovered and remained in good health for at least one month. The employment of other temperatures ranging from 28 to 41°C. yielded no encouraging results, but the number of these tests was smaller.

[4] That closely associated centers are powerfully stimulated is evident from the accompanying vomiting, dyspnoea, and vagal involvement. Interference with central heat regulation by peripheral nerve irritation was long since suggested by H. C. Wood, who concluded from experiments upon dogs that "galvanization of a sensitive nerve produces a fall of the bodily temperature by acting upon some nervous center situated either in or above the pons." Smithsonian Contributions to Knowledge, 1881, xxiii, no. 357, p. 91.

[5] F. P. Underhill, Loc. cit. Cf. also: Woodyatt, R. T., Arch. Int. Med., 1919, Jul. 15.

CONCLUSIONS

1. Low (irritant) concentrations of chlorine (24 to 30 parts per million of air) produce a considerable increase in the body temperature of dogs.

2. Medium (irritant-depressive) concentrations of chlorine (180 to 200 parts per million) produce a considerable decrease in temperature. Marked retardation of the pulse rate also occurs. Both phenomena begin to disappear as soon as the half-hour gassing is over.

3. Lethal concentrations of chlorine (800 to 900 parts per million) have a similar effect upon the temperature curve to that of medium concentrations but it is distinguished from the latter by the persistence of the fall after gassing. The slowed pulse becomes weak and rapid within a few hours and collapse ensues.

4. Neither the hyperthermia nor the hypothermia described bear any direct relationship to variations in the rate of respiration or of the heart.

5. When long haired dogs (collies) are gassed with lethal concentrations the picture is varied by the occurrence of marked hyperthermia persisting for about two hours, followed by an early collapse and death.

6. Dogs gassed with lethal concentrations of chlorine tend to become poikilothermic, being unable to regulate against either a moderately warm or a moderately cool environment.

7. Inhibitory effects of peripheral nerve irritation upon the temperature centers may contribute substantially to the derangement of the heat regulating mechanism seen with all of the concentrations of chlorine employed.

8. In the treatment of gassed cases it is suggested that an excessively warm environment may be as dangerous as one which is too cold.

The author acknowledges with thanks much valuable assistance rendered by Messrs. E. Fisher, R. R. Killinger, C. R. Scott and F. A. Taylor.

ON THE PENETRATION OF DICHLOROETHYLSULPHIDE (MUSTARD GAS) INTO MARINE ORGANISMS, AND THE MECHANISM OF ITS DESTRUCTIVE ACTION ON PROTOPLASM

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INTRODUCTION

The present investigation[1] was undertaken at the request of Major H. C. Bradley, of the Chemical Warfare Service, the purpose being to determine the nature of the mechanism whereby "mustard gas" (dichloroethylsulphide) penetrates and causes destructive effects on living tissues.

[1] This report of work completed in September, 1918, and submitted to the Chemical Warfare Service, has been released for publication.

By utilizing the developing eggs of starfish, sea urchins and other marine animals, it was hoped that light might be thrown on the general nature of the abnormal changes produced in protoplasm, i.e., in the essential living substance itself considered independently of the special peculiarities of the organism to which it belongs.

Mustard gas, in common with the majority of effective war gases, is relatively freely soluble in organic solvents and only sparingly soluble in water, but its aqueous solutions undergo hydrolysis with the production of nascent hydrochloric acid according to the equation

$$(C_2H_4Cl)_2\, S + 2H_2O = (C_2H_4OH)_2\, S + 2H\, Cl.$$

The velocity with which this reaction proceeds differs widely for different war gases. In the case of "mustard," 50 per cent of a saturated aqueous solution is hydrolyzed in ten minutes at 20°, and in one and one-half to two minutes at 37.5°.

Since strong mineral acids and bases do not appear to penetrate protoplasm with facility, but exert their destructive effects primarily on the exterior portions of the cell, while weak acids and bases and substances like anaesthetics, possessing a relatively higher solubility in organic reagents, penetrate protoplasm with facility, it appears probable that "mustard" and other war gases exert their toxic effects in virtue of two distinct properties: one, their organo-solubility, facilitating their ready passage into the interior of the cell; two, the facility with which they undergo hydrolysis in contact with water, leading to the production of nascent hydrochloric acid within the cell, where the destructive effect is obviously far greater than that exerted by a corresponding amount of acid on the exterior.

This view regarding the mode of action of "mustard gas," has been advocated by Marshall (1), Smith (2) and other investigators on the basis of physiological and pharmacological experiments, but no absolutely direct evidence has thus far been forthcoming that hydrochloric acid in the interior of the cell would produce effects analogous to those exerted by "mustard gas" which has penetrated from without.

The extraordinary toxicity of "mustard gas" and the prolonged latent period which elapses before destructive effects are exerted appeared at first sight to present difficulties in the acceptance of the above theory, but it should be remembered that a substance like "mustard," if it can be demonstrated to penetrate the cell with facility, would presumably be dissolved in, adsorbed by or combined with lipoidal and other constituents of the cell. If some type of specific selective action be postulated, it is conceivable that the very minute traces of nascent hydrochloric acid produced would disturb the delicately balanced equilibrium of the substances in question to an extent sufficient to cause necrosis and death of the cell.

As regards the prolonged latent period of several hours, compared with the known rate of hydrolysis of "mustard gas" in water at body temperatures, it is quite obvious that this difficulty might be explained in one of two ways: either the mustard is held bound in lipoidal or other nonaqueous constituents of the cell and only released slowly into the water phase, where it undergoes hydrolysis, or "mustard gas" is actually chemically combined with protoplasmic complexes possessed of such dimensions that the rate of hydrolysis and consequent production of acid is considerably retarded. Numerous analogies for this point of view may be found in organic chemistry.

It must also be borne in mind that the resistance of the buffer system regulating the hydrogen ion content of protoplasm must first be overcome before the acid concentration can reach that critical level at which a disturbance of chemical or colloidal equilibrium of protoplasmic constituents is induced sufficient to impair or destroy vital function.

The theory that the toxic action of "mustard gas" is attributable to ionizable chlorine, causing oxidative effects, finds no support from data thus far available. There is no evidence that mustard or any of the other war gases produce $HClO$ instead of HCl on hydrolysis in either physical or biological systems.

LIVING MATERIAL EMPLOYED IN THE INVESTIGATION

Since the purpose of this investigation was to determine the mode of penetration and toxic action of war gases in living protoplasm, and the possible means of counteracting their effects, most of the experiments were conducted upon living material of structurally very simple character, for example, the freshly fertilized eggs of the starfish or sea urchin. These eggs are single cells at the beginning of development, are uniform in size and properties, and are readily obtainable in large quantities. They exhibit certain definite and constant peculiarities of appearance and behavior by which an accurate index of the rate and character of the toxic action of a given substance may readily be obtained.

The physiology of the eggs of starfish and sea urchins has been studied extensively both at Woods Hole and elsewhere, and the normal properties and behavior of the eggs were well known at the beginning of the investigation, as were also many of the modifications exhibited under special experimental conditions (variations in temperature, acid-alkali reaction and saline content of the medium; presence of various toxic compounds, anaesthetics, etc., which may be introduced into the medium). The abnormalities of appearance and behavior induced by exposure to "mustard gas" and various other substances, when compared with effects previously recognized, afforded information regarding the mode of action of the war gases.

Other living material employed included spermatozoa of starfish and sea urchins, free-swimming larvae of various kinds, including annelids (Nereis, Arenicola), echinoderms (starfish, sea urchin), and squid, and young and adult fish (Fundulus). The experiments with these latter forms were less extensive than those with developing eggs, and served mainly for corroboration and control.

It appears desirable at this stage to include a somewhat detailed description of the normal development of starfish eggs, the material principally employed in these experiments, for the information of chemists, pharmacologists and others who are inter-

ested in the action of war gases but are at present unacquainted with this marine material and the means which may best be employed to utilize it for investigations of this type.

STARFISH EGGS

The starfish at Woods Hole (Asterias forbesii) are of separates sexes, externally similar in appearance. The eggs are readily obtained from the females by detaching the arms, removing the ovaries with forceps, and transferring these to dishes filled with sea water (finger-bowls of 300 cc. capacity were used). The ripe eggs detach themselves spontaneously from the ovaries, a process assisted by shaking; the ovaries are then removed from the dishes and the eggs are washed by two or three changes of sea water; they are then in condition for experimentation. Starfish produce eggs at Woods Hole from May to September. The breeding season is at its height in May or early June; later in the summer the eggs are usually fewer and less uniform in quality, but in 1918 good material was obtained until nearly the middle of September. The freshly removed eggs are yellow to light pink in color when seen in mass; the single egg is a spherical cell of naked translucent protoplasm, emulsion-like in appearance and consistency, and has a sharply defined contour and a definite surface-film denser in structure than the internal protoplasm; the diameter is about 150 microns. The freshly laid egg contains a large clearly defined spherical nucleus or "germinal vesicle," with a clearly visible single nucleolus. After standing for several minutes in normal sea water the nuclear membrane gradually fades away and the nucleus becomes invisible in the living egg; this process is the preliminary stage of maturation (the cytological change preparatory to fertilization) without which further development is impossible.

Since the work with starfish eggs consisted chiefly in a study of the action of solutions of "mustard gas" in sea water upon the cleavage and early development, fertilized eggs were generally employed. The eggs are fertilized artificially as follows: the testes are removed from the animal and placed in dishes of sea water separately from the eggs. The spermatozoa (each of which is a minute flagellate cell with rounded head and slender vibratile tail) are rapidly freed and form a milky suspension in the sea water; a few drops of this suspension, stirred into a dish of sea water containing mature eggs, will fertilize all in a few minutes. In fertilization a single spermatozoon unites with each

egg; the first visible change in the latter is the separation of a sharply defined thin membrane, the fertilization-membrane, from the egg surface. After fertilization, the eggs are freed from superfluous spermatozoa by washing in two or three changes of sea water; they are then ready for use.

Fertilization is followed after a definite interval by cleavage, which consists in a progressive subdivision of the egg into numerous smaller cells; these are at first similar in size and appearance; later they undergo differentiation and rearrangement to form the early tissues and organs of the developing animal. In the starfish egg under normal conditions cleavage is regular and equal; the originally spherical cell divides into two equal cells by indirect or mitotic cell-division; each of these daughter-cells again divides equally, forming a group of four cells; the third division bisects each of these, forming 8 cells; and by a similar succession of equal divisions the egg passes through 16-, 32-, 64-, 128- and later cell-stages. The cleavages are at first synchronous in the different cells of the same cell-generation; later divergences of rate appear in different cells. The original single egg-cell is thus by degrees transformed into a spherical mass of numerous small cells; as cell-multiplication continues these undergo rearrangement, forming about eight hours after fertilization (at 20°) a hollow sphere with a central cavity (blastocoel) and a wall of a single layer of cubical cells. The outer surfaces of these cells soon acquire cilia which cause the whole structure (now in the early "blastula" stage) to rotate within the fertilization-membrane. These earlier developmental stages are passed through while the egg is still enclosed by this membrane; the latter now soon disappears, and by ten hours after fertilization the blastulae are swimming freely in the sea water. Normal blastulae and gastrulae are negatively geotropic and swim at the surface, although specifically heavier than sea water. The blastula, at first a single-walled spherical vesicle, soon becomes somewhat elongated or ovoid in shape, and is then converted into the next or so-called "gastrula" stage by the inpocketing or "invagination" of a portion of its wall; this change begins at twelve to sixteen hours after fertilization (at 20°) and gives rise to a symmetrical sac-shaped larval organism with an inner blind tube (archenteron), formed from the invagination and open to the exterior, and an outer wall (ectoderm) merging into the archenteron at the margin of the opening (blastopore). The archenteron (entoderm) forms the later larval intestine. By further transformation of the gastrula a typical bilateral swimming larva, the so-called

Bipinnaria, is formed; at forty-eight hours after fertilization (at 20°) the young Bipinnaria is well developed. By a somewhat complex metamorphosis the Bipinnaria gives rise to the young star fish.

The normal time-relations of the cleavage-process and early development are briefly as follows: Eggs fertilized in normal sea water at the most favorable time (thirty to forty minutes after removal from the animal) begin the first cleavage (at the normal summer temperature of the sea water, about 20°) about one and one-half hours after fertilization, i.e., about thirty minutes after the separation of the second polar body; the second cleavage follows about forty to forty-five minutes later, and the intervals between the next few cleavages are similar (thirty-five to forty-five minutes). Four hours after fertilization such eggs are typically found in a stage of about 64 cells of uniform size.

The rate of cleavage is influenced by various external conditions. Cleavage is temporarily arrested by removal of oxygen, presence of cyanide, anæsthetics, or a sufficiently low temperature. The temperature coefficient of the process is high (Q_{10} = 2–4), so that in order to secure a constant rate of cleavage the temperature, as well as the oxygen-content, reaction and composition of the medium, must be kept constant.

Under normal conditions at 20° the eggs reach the ciliated blastula stage in about eight hours after fertilization The ciliary movement is vigorous and begins before the blastulae are set free, causing them to rotate actively inside the fertilization-membrane. Soon afterwards the membrane becomes thinner, apparently by some progressive solvent action, and disappears; the blastulae then swim actively and rise to the surface of the water (negative geotaxis); gastrulation begins about twelve hours after fertilization and is complete a few hours later. It should be understood that on account of the high temperature-coefficient of the developmental process (Q_{10} = about 3), the time required to reach any stage in eggs developing in dishes is greatly influenced by the temperature of the room. This brief sketch of the early development is necessary to make clear the nature of the abnormalities of structure, development and behavior induced by "mustard gas" in the eggs. For further details regarding development, the reader is referred to the text-books of comparative embryology (e.g., E. B. Wilson, Cell in Development and Inheritance; Korschelt and Heider, Text-book of Comparative Embryology).

ACTION OF SOLUTIONS OF "MUSTARD GAS" ON STARFISH EGGS

Eggs which have been exposed to "mustard gas" (or other poisons) show various definite abnormalities of cleavage and development, followed by production of irregular or otherwise abnormal larval forms to be described below.

The poisoned eggs show a retarded rate of cleavage and development, the degree of retardation varying directly with the degree of poisoning, i.e., with the time of exposure to a given solution of "mustard gas." Cleavage also tends to be irregular in such eggs, and these irregularly cleaving eggs give rise to irregularly shaped or asymmetrical blastulae and gastrulae. If the degree of poisoning is sufficient, the eggs entirely fail to cleave, and disintegrate within a few hours. With less severe poisoning they may cleave several times, usually irregularly and at a retarded rate; cleavage then ceases and a gradual breakdown follows. With slight poisoning development may proceed to the blastula or early gastrula stage, but more slowly than normally; these larvae are typically irregular, thick-walled, or undersized, and their ciliary movement is too weak to carry them to the surface of the water. The proportion of eggs forming larvae capable of swimming at the surface of the water is a good index of the degree of toxicity, if the poisoning is not too severe; a high proportion of surface-larvae always indicates a slight toxic action. This index is used in many of the experiments described below.

The work with starfish eggs was begun July 31, and was continued until September 9, when the breeding season was near its close and good eggs were difficult to obtain.

Methods. A solution made by dissolving a definite quantity of "mustard gas" in sea water varies in its composition according to the time that has elapsed since the solution was first made, and its toxic properties decrease correspondingly, because of the rapid hydrolysis which the compound undergoes in aqueous solution. In the earlier experiments with starfish eggs, a small quantity of the "mustard gas" (about 5 grams) was shaken vigorously with one liter of sea water in a 2-liter glass-stoppered bottle. After the

finely divided undissolved "mustard gas" had settled, the clear liquid from the middle of the solution was drawn off, and the action of this solution upon the recently fertilized eggs was tested, using varying dilutions (e.g., $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ saturated) and varying times of exposure (from one-quarter minute to an hour or more). The eggs were exposed to these solutions in glass-stoppered bottles, and at intervals portions of the eggs were transferred by pipette to dishes of normal sea water; this water was changed when the eggs had settled. The subsequent course of development, as compared with that of untreated "control" eggs, was carefully studied.

Considerable irregularity was observed in the toxicity of different solutions, prepared similarly and used at similar intervals after preparation. Since such solutions were usually not quite clear, it seemed likely that this irregularity was to be referred to the presence of variable quantities of finely divided undissolved mustard gas in suspension. The problem thus arose of how to prepare solutions free from these accidental particles.

"Mustard gas" solidifies at about 10°C., and the solid crystals can very readily be filtered off from the aqueous suspension. The procedure finally adopted to secure saturated solutions, absolutely free from suspended "mustard" particles, was as follows: sea water was cooled to 0°C., "mustard gas" was introduced drop by drop into the ice cold water and the mixture vigorously shaken. The mustard crystallized, and after a brief period of shaking the undissolved crystals were filtered out, precautions being taken to maintain the saturated solution of mustard in sea water at a temperature of 0°C. The rate of hydrolysis of this solution is comparatively slow, but in the course of twenty-four hours its toxic action had diminished more than half, and in the course of forty-eight hours more than three-quarters. To carry out experiments with marine eggs, the ice cold solution was brought to the temperature required by the experiment, by dilution with sea water at a higher temperature, immediately before introducing the eggs. When it was desired to make a comparison between the undecomposed "mustard" solution and one which had stood at room temperature for a given length of

time, the saturated ice cold solution of "mustard gas" was diluted with warm sea water so as to bring it to the working temperature at whatever interval of time was desired prior to the commencement of the experiment.

The concentration of unaltered "mustard gas" in a freshly prepared saturated solution at room temperature, was found to be approximately 0.05 per cent (as estimated by titration of completely decomposed solutions). The concentration rapidly decreases at room temperature as the compound hydrolyzes. Experiments, one of which is depicted subsequently in figure 2, p. 24, indicate that the decline in the toxicity of the solution runs closely parallel with the progress of decomposition.

The rate of decomposition was determined by titrating, at intervals, the hydrochloric acid produced in the solution. When great accuracy was required, saturated solutions were prepared in distilled water and titrated against their loss of toxicity. The velocity of hydrolysis is a function of the temperature. When the solution is brought to room temperature, the decline in toxicity is at first rapid, subsequently becoming more gradual, the curve corresponding with the mono-molecular curve of hydrolysis. From a large variety of experiments there is little doubt that the essential toxic agent in the solution is the undecomposed "mustard gas." Under normal conditions of temperature, etc., the toxicity of a saturated sea water solution is diminished to one-half in less than fifteen minutes, to one-fourth in less than thirty minutes, one-eighth in less than an hour, and only a very small residual toxicity is recognizable after twenty-four hours.

Since the experiments were conducted under such conditions that the hydrochloric acid produced was neutralized by the buffer system of the sea water, the toxic action of this solution after twenty-four hours was not attributable to the hydrochloric acid. From separate experiments with di-hydroxy-ethyl-sulphide, the other product of hydrolysis, there is reason to believe that it is entirely non-toxic. The small residual toxicity exhibited by solutions, even after standing for twenty-four hours, is a matter of considerable interest and suggests the probability that eggs and other protoplasmic structures possess a strong adsorptive

capacity for even the minute traces of the gas still remaining undecomposed in the solution.

The action of "mustard gas" is so entirely different from the action of hydrochloric and other acids applied externally, as to eliminate any likelihood of confusion resulting from toxic effects exerted by externally liberated acid in those cases in which the sea water was rendered slightly acid prior to the termination of the experiments.

For reasons of space it seems best not to describe a large number of experiments in detail, but to give first a complete description of those experiments which were carried out with the best technique and material and gave the clearest results. The other experiments all confirmed these results, and furnished additional details of greater or less interest which will be described briefly later. The order of description is thus not chronological, but is chosen so as to bring out most clearly the essential facts established in the investigation.

In the following experiments the eggs were placed, usually within thirty to forty-five minutes after fertilization, in the solution whose action was being tested. After remaining in this solution for the definite time chosen for the exposure, they were returned to normal sea water. Later the course of cleavage and development was observed and compared with that of the control lot of untreated eggs. The "mustard" solutions used were contained in glass-stoppered wide mouthed bottles of about 20 cc. capacity. The same volume of solution, from 10 to 20 cc., was used in each experiment of a series. The eggs were transferred from the normal sea water to the "mustard" solutions and back by means of wide-mouthed rubber-bulb pipettes ("medicine-droppers"), care being taken that the volume of eggs plus sea water introduced into each solution of a series was constant (usually about 3 cc.). Usually in each series three or four different solutions were under comparison (differing in composition, dilution or otherwise); eggs were placed in each solution of the series at about the same time, and after fixed intervals of time, ranging from one minute to thirty-two minutes, portions of the eggs were transferred by pipette from the "mustard" solutions

to normal sea water; about 300 cc. of sea water contained in a finger bowl was used for each portion of exposed eggs. Two observers coöperated in the manipulation; one making the transfers of eggs between sea water and solutions, and the other reading the times at which the transfers were to be made. With, e.g., four solutions to a series and brief intervals between the successive transfers coöperation of this kind proved necessary.

The variables in treatment were thus (a) character of solution used and (b) time of exposure to solution. All exposures were made with the solution at room temperature, except in those experiments where the influence of temperature on the rate of toxic action was itself under investigation. With strongly toxic solutions very brief exposures (of less than a minute) are sufficient to kill all of the eggs within a few hours. In such cases the toxic effect is not immediately apparent but makes itself evident later, i.e., a prolonged latency is characteristic of the action of "mustard gas" upon these eggs, as well as upon the tissues of higher organisms.

The first series of experiments to be described (table 1) illustrates the rapid decline in the toxicity of the freshly prepared ice-cold solution after bringing to room temperature.

The order of relative toxicity in the above series is clearly $A < B < C < D$. The difference between A and B is slight but distinct; note, e.g., the greater retardation in development in the B series as shown by the delay in freeing the blastulae from the fertilization-membranes. The difference between B and C is somewhat greater than that between A and B, and that between C and D is the greatest. That is, the decline in toxicity is distinctly greater during the first fifteen minutes than during the second fifteen minutes after bringing the mustard solution to room temperature. An exposure of one minute to solution D prevents all eggs from forming blastulae having sufficient ciliary activity to swim at the surface; to produce the same effect with solution A, two to four minutes exposure is required, and two minutes with solution B. Similar differences are seen if the comparison is made on the basis of the proportion of poisoned eggs remaining alive after forty-five hours in sea water; there are

TABLE 1

September 9, 1918. A cold saturated solution of "mustard gas" was made at 11.30 a.m. by shaking several grams of the oil with a liter of cold sea water (about 4°) in a 2-liter bottle. The bottle was then kept surrounded by ice in the ice-box and shaken several times at intervals up to 12.30. At 2.15 this cold solution (containing solidified finely divided "mustard" in suspension) was filtered through an ice-cold filter into a bottle surrounded by ice; this gave a clear solution quite free from suspended particles. This solution was used in one-third saturated concentration (1 volume saturated solution plus 2 volumes sea water). The action of four solutions (A, B, C, D) upon the eggs was tested. These solutions differed only in respect to the time during which they were kept at room temperature before adding to the eggs. The solutions were taken from the ice, brought to room temperature and diluted for use at the following times previously to the experiment.

Solution A warmed to 19° and diluted at 2.28 to 2.30 and kept at room temperature until 3.31, when it was added to the eggs.

Solution B brought to room temperature and diluted at 2.55 to 2.57; added to eggs at 3.31½.

Solution C brought to room temperature and diluted at 3.10 to 3.11; added to eggs at 3.26.

Solution D. Brought to room temperature and diluted immediately (within 1 minute) before adding to eggs at 3.26½ (the dilution and warming were accomplished by adding two volumes of sea water warmed to about 30° to one volume of cold "mustard" solution, giving a mixture of about 20° temperature).

The starfish eggs were removed from the animals at 2.30 p.m., fertilized at 3.05, and placed in the solution at about 20 to 25 minutes after fertilization. Eggs were transferred from each solution to sea water after exposures of 1, 2, 4, 8 and 16 minutes. The results for each solution were as follows:

Solution A. One-third saturated "mustard." Solution at 0° for about 3 hours (11.30 to 2.28), then at room temperature (19 to 20°) for about 1 hour (2.28 to 3.31).

Eggs placed in solution at 3.31. The condition of the eggs next morning (after 18 to 20 hours in sea water) was as follows:

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT
	min.	
1	1	After 18 hours nearly all eggs have formed blastulae already free from fertilization-membranes; later a large proportion swim at the surface of the sea water. Most are still living after 45 hours.
2	2	At 18 hours nearly all eggs are in blastula stage, most still inside fertilization-membranes; later the larvæ swim freely but few rise to the surface. Most are still alive after 45 hours, but abnormalities are more frequent than in Experiment 1.

TABLE 1—*Continued*

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT
	min.	
3	4	Most eggs are blastulae at 18 hours, inside membranes, more irregular in shape and feebler in movement than in experiment 2. No surface swimmers. Nearly all dead at 45 hours.
4	8	Contrast to experiment 3. 50 per cent are disintegrated at 18 hours, remainder are irregular feeble blastulae inside membranes. No surface-swimmers; all dead and disintegrated at 45 hours.
5	16	At 18 hours nearly all eggs are dead and disintegrated; an occasional abnormal living blastula inside the membrane.
Solution B. Same mustard solution: at 0° from 11.30 to 2.55; then at room temperature for about 36 m. (2.55 to $3.31\frac{1}{2}$). Eggs placed in solution at $3.31\frac{1}{2}$.		
1	1	At 18 hours the great majority of eggs have formed blastulae as in A-1, but about 50 per cent are still inside membranes (difference from A-1); only a few rise later to the surface. Most are living at 45 hours but are less favorable than in A-1.
2	2	At 18 hours most are more or less irregular blastulae inside membranes. No surface swimmers. A large proportion are dead at 45 hours, though some feeble abnormal forms remain.
3	4	At 18 hours about one-third of the eggs are disintegrated; the remainder are more or less irregular blastulae inside membranes. No surface swimmers. At 45 hours almost all are dead and disintegrated, but a few are living.
4	8	At 18 hours nearly all show well-marked disintegration, but many form partial or otherwise irregular blastulae inside membranes. All are dead at 45 hours.
5	16	Nearly all are dead and disintegrated at 18 hours; an occasional living form remains (fewer than in A-5).
Solution C. Same solution; at 0° from 11.30 to 3.10; at room temperature about 16 m. (3.10 to 3.26). Eggs placed in solution at 3.26.		
1	1	At 18 hours most eggs have formed blastulae as in A-1 and B-1, but almost all are still inside membranes. Practically none are at surface (difference from A-1 and B-1). At 45 hours most are feeble undersized blastulae, but still living.
2	2	At 18 hours about 60 to 70 per cent are blastulae inside membranes; disintegrated eggs are more numerous than in B-2. At 45 hours the great majority are disintegrated; a few irregular blastulae survive.

TABLE 1—*Concluded*

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT
	min.	
3	4	At 18 hours 40 to 50 per cent are disintegrated; the rest are irregular blastulae inside membranes; abnormalities are more marked and frequent than in B-3. All are dead and disintegrated at 45 hours.
4	8	Disintegration is more marked than in B-4; larvae are less frequent and are mostly irregular or partial. All are dead at 45 hours.
5	16	Complete disintegration at 18 hours; none then living.

Solution D. Same solution kept cold from 11.30 a.m. until immediately before using. Eggs placed in solution at 3.26½.

1	1	Most eggs form blastulae by 18 hours but these are distinctly less favorable than C-1 (cilia less active, more undersized or partly disintegrated forms). No surface swimmers. Most are still alive at 45 hours.
2	2	Partial disintegration in about 50 per cent eggs at 18 hours. Ciliary movement is feeble. All are dead at 45 hours.
3	4	Most eggs are disintegrated at 18 hours. Some partial or irregular blastulae remain alive. All are dead at 45 hours.
4	8	Contrast to experiment 3; at 18 hours 90 per cent or more are dead and disintegrated. A few partial or irregular blastulae remain living. All are dead at 45 hours.
5	16	At 18 hours all are dead and disintegrated.

Control eggs. At 18 hours the surface of the water is swarming with blastulae just beginning to gastrulate.

a few survivors from the four minute exposure in series A and B (B < A), none in C (although a good many survive two minute exposures), while in series D all eggs exposed for two minutes are dead at forty-five hours. The effect produced by two minutes in solution D is approximately equal to that produced by eight minutes in solution A. As nearly as can be estimated, solution D is three to four times more toxic than solution A, as indicated by the time of exposure required to produce a definite degree of toxic effect.

It has been found that at 0° the decline of toxicity is gradual, requiring about twenty-four hours to reach one-half to one-third

its initial value. In the solutions of table 1, the change in toxicity thus took place almost entirely within the period during which the solution was at room temperature. A saturated solution kept at 0° for twenty-four hours is still markedly toxic (cf. table 2, solution D), while one kept at room temperature for the same time has only slight action; after 44 hours at room temperature the toxicity has almost completely disappeared (cf. table 3, solution A).

The effects of more prolonged exposure to room temperature upon the toxicity of "mustard" solutions are illustrated by tables 2 and 3, which give a summary of similar experiments with solutions prepared in the cold and kept at room temperature for varying periods.

In the series of table 2 the order of relative toxicity is again A < B < C < D. Comparisons between the effects of equal exposures to different solutions show again that the toxicity declines on standing at room temperature, at first rapidly, then more slowly. The difference between solutions D and C is decidedly greater than between C and B, although the exposure to room temperature was more than twice as long in B as in C. If any single character is taken as a basis of comparison (e.g., the presence of swimming larvae at the surface), solution D appears to be about eight times as toxic as solution C and at least sixteen times as toxic as solution A, i.e., an exposure of one minute to solution D has essentially the same effect as an exposure of eight minutes to C or sixteen minutes (at least) to A. A diagram (fig. 1) may indicate these differences more clearly.

Any exposure represented by a square the whole or part of which is above the diagonal fails to poison the eggs sufficiently to prevent the formation of normally swimming larvae. Two minutes exposure to D has this degree of toxic effect, while sixteen minutes exposure to A leaves a fair proportion of eggs still capable of forming surface larvae.

Still longer exposure of the "mustard" solution to room temperature deprives it of all but a trace of toxicity. A solution exposed at 20° for two days requires, under the same conditions as those of tables 1 and 2, more than forty-five minutes to pro-

TABLE 2

September 6. A saturated solution of "mustard gas" in ice-cold seawater was made September 5, at 10.30 a.m. The clear filtered solution was kept at 0° in the refrigerator, portions being removed and brought to room temperature at the times indicated.

The solutions used were as follows:

A. Solution brought to room temperature 2.30 p.m., September 5, kept at room temperature until used next day at 11.10, i.e., for about 20 hours.

B. Solution brought to room temperature 7.45 a.m., September 6, kept at room temperature for about 3½ hours before using.

C. Solution warmed to room temperature about 1 hour 30 minutes before using.

D. Solution kept at 0° until immediately before using.

Each solution was used in one-third dilution as before. (This dilution with sea water is sufficient to neutralize the acid freed in the hydrolysis of the "mustard gas," so that the effect of the solution is due entirely to the dissolved gas and the decomposition products other than acid). The starfish eggs were placed in the solutions about 30 minutes after fertilization, and received the same exposures as in table 1, viz., 1, 2, 4, 8 and 16 minutes. In the description of results a statement of the condition of the eggs at a time 3½ to 4 hours after placing in the solution (at which time the normal eggs are in the 64 to 128-cell stage), and again the next day (after about 23 hours) is given.

Solution A. One-third saturated "mustard." Solution at 0° for about 4 hours (10.30 to 2.30, September 5), then at room temperature for about 20½ hours (2.30 September 5 to 11.10 September 6). Eggs placed in solution at 11.10, September 6, 30 minutes after fertilization; portions transferred to seawater after the following exposures:

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT
	min.	
1	1	Cleavage is not evidently retarded. Next day the surface is full of active swimmers.
2	2	Essentially like experiment 1.
3	4	Cleavage is well advanced. Numerous surface larvae.
4	8	Cleavage is on the whole less advanced and less regular than in experiments 1 to 3. Surface larvae are fewer.
5	16	Most eggs at 4 hours are in 16 to 32 cell stage and many have cleaved irregularly or have undergone irregular change of form without definite cleavage. Surface larvae are few.

Solution B. At 0° about 21 hours, at room temperature about 3½ hours (7.45 to 11.10½, September 6).

1	1	No evident effect on cleavage. Numerous surface larvae.
2	2	Cleavage is somewhat retarded (most in 32 to 64 cells); many surface larvae next day.

TABLE 2—*Concluded*

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT
	min.	
3	4	Cleavage is retarded and less advanced on the whole than in experiment B-2; a good many surface larvae next day.
4	8	Retardation of cleavage is well-marked and cleavage is less regular than in experiment 3; surface larvae few (similar to experiment A-5).
5	16	Cleavage is less advanced than in A-5, and many eggs are of irregular form and uncleaved; cleavage stages are very irregular, mostly 8 to 16 cells. No surface swimmers present next day (difference from A-5).

Solution C. At 0° about 23 hours, at room temperature about 1½ hours (9.30 to 11.05 a.m., September 6).

1	1	Cleavage shows some retardation. A good many surface larvae.
2	2	Cleavage is less advanced than in C-1 or B-2; surface swimmers as in experiment C-1.
3	4	Cleavage is mostly 8 to 16 cells (less advanced than in C-2); fewer surface larvae.
4	8	Cleavage is mostly 8 to 16 cells, with some 4-cell stages (retardation > C-3 or B-4). Few surface swimmers (< B-4 or C-3).
5	16	Cleavage is irregular and greatly retarded; many uncleaved eggs, others in 4, 8, and 16 cells. No surface larvae present.

Solution D. Kept cold until immediately before using (i.e., kept about 24½ hours at 0°).

1	1	Cleavage shows decided retardation; the eggs are mostly in 16 to 32 cell stages, and many irregular. Only a few surface larvae—very like C-4 or B-4.
2	2	Most eggs are in irregular 16 to 32 cells with some 8- and 4-cell stages. No surface larvae present. Numerous larvae at bottom.
3	4	Cleavage is more retarded than in D-2, largely irregular 16-cell stages with many 8 cells. Decidedly behind C-3. No larvae at surface.
4	8	Retardation of cleavage is marked; many irregular 8 cells and some uncleaved eggs. No surface larvae. Most eggs at bottom of dish are disintegrated.
5	16	About 35 to 50 per cent of eggs are uncleaved and largely irregular in form. Others show irregular cleavage groups up to 16 cells.

Control. The untreated eggs develop normally. The surface of the seawater is full of active normal gastrulae next morning.

duce a well-marked toxic effect; and even this exposure is insufficient to prevent a considerable proportion of eggs from forming surface larvae. Table 3 gives a description of a further series of experiments with the same solution as that used in the experiments of table 2, performed on the day following (September 7).

The order of relative toxicity is again A < B < C < D, but the absolute toxicity has declined greatly since the preceding day. Solutions A and B show little appreciable action with sixteen minutes' exposure, and even after thirty-four minutes' treatment most eggs form larvæ, some of which swim at the

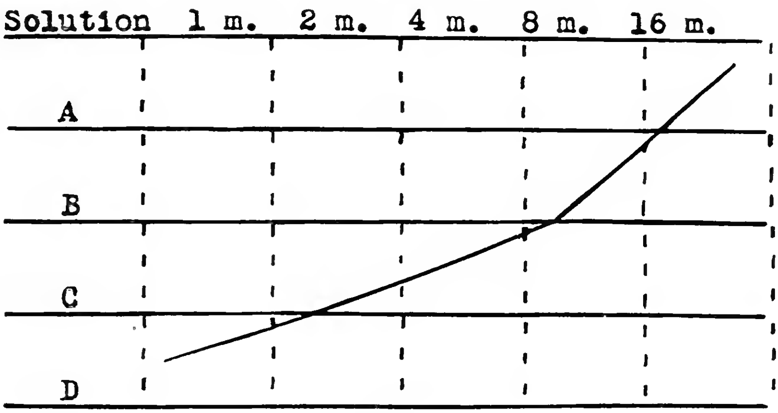

Fig. 1

surface. Solutions A and B exhibit little difference from each other; C shows considerably greater toxicity than B, while D is decidedly the most toxic. This solution (D of table 3) after keeping cold for forty-four hours, is decidedly less toxic than it was on the preceding day (solution D of table 2) after remaining only twenty-four hours in the cold. The decline of toxicity proceeds at 0° much more slowly than at room temperature. The rate of decline at 0° may be estimated by comparing the effects of solution D in the three tables. In the experiments of table 1, solution D had been prepared at 0° and kept four hours at 0° before using; four minutes in this solution produced

TABLE 3

September 7. The same solution (prepared September 5) was used as in the series of table 2. The cold filtered solution was kept surrounded by ice in the refrigerator, and portions were removed at the times indicated, warmed to room temperature, kept at room temperature for the periods indicated, and their action in the eggs tested as before (dilution one-third as in table 2). The fertilized eggs were placed in the solution about 30 minutes after fertilization.

Solution A. Filtered solution, one-third dilute, removed from ice September 5 at 3.30 p.m. and kept at room temperature for about 44 hours before adding the eggs.

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT
	minutes	
1	1	Cleavage after $3\frac{1}{2}$ hours is the same as in control. After 22 hours the surface is full of active blastulae and gastrulae.
2	2	Similar to A-1.
3	4	Little effect, slight retardation; surface larvae as in A-1 and 2.
4	8	Little difference from A-3.
5	16	Cleavage is somewhat less advanced and regular than in A-4. Many surface larvae.
6	about 34	Here there is well-marked retardation and inhibition of cleavage. Surface larvae are fewer though still quite numerous.

Solution B. Solution on ice September 5 to 6; kept at room temperature for about 20 hours before adding the eggs.

1	1	Cleavage is like control. Many surface larvae.
2	2	Also shows little effect. Surface is full of larvae next day.
3	4	Very like A-3; slight retardation, many surface larvae.
4	8	Cleavage is somewhat retarded as in A-4; numerous surface larvae.
5	16	Very like A-5; surface larvae as above.
6	34	About 50 per cent of eggs are uncleaved at $3\frac{1}{2}$ hours; the rest are cleaved but are largely retarded or irregular. Still a good many surface larvae, as in A-6.

Solution C. Kept on ice September 5 to 7; warmed to room temperature about 40 minutes before adding eggs.

1	1	Cleavage is somewhat less advanced than in B-1. Many surface larvae next day.
2	2	Essentially like C-1.
3	4	Some retardation is apparent. Surface larvae many.
4	8	Cleavage is less advanced than in C-3, mostly in 8-cell; many surface larvae.
5	16	Here cleavage stages are largely 4-cell (control is 16 to 32 cell) with many 8-cell; less advanced than C-4. Surface swimmers are many but relatively fewer than in C-4.
6	34	Here a large proportion of eggs are uncleaved (about 70 to 80 per cent); inhibition > B-6. Almost no surface larvae. Many blastulae are living next day at bottom of dish.

TABLE 3—*Continued*

Solution D. Solution kept on ice about 44 hours, until immediately before using.

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT
	minutes	
1	1	Cleavage is well advanced at $3\frac{1}{2}$ hours. Many surface swimmers next day.
2	2	Cleavage is somewhat less advanced than in D-1; 8-cell stages are more frequent. Many surface larvae.
3	4	Proportion of 8-cells is greater than in D-2; surface larvae many.
4	8	Most eggs are in 8-cell at $3\frac{1}{2}$ hours, less advanced than in C-4. Surface larvae many.
5	16	At $3\frac{1}{2}$ hours about 30 to 40 per cent are uncleaved; the rest are largely irregular 4- and 8-cell groups. Falling off in surface larvae; few are present; many blastulae at bottom of dish.
6	34	Almost all eggs are uncleaved, a few partial or irregular cleavages; few eggs are living next day; no surface larvae; a few irregular blastulae at bottom of dish.

Control. Great majority of eggs cleave and develop normally. Dish full of active gastrulae next day.

approximately the same effect as eight minutes in the solution which had been kept for twenty-four hours at 0° before using (D of table 2). More than sixteen minutes was required to produce this effect on the day following (D of table 3). That is, in twenty-four hours at 0° toxicity decreases by about one-half. The observations of table 1 show that at room temperature (20°) a similar proportional decline in toxicity requires less than half an hour (compare effects of solutions B, C, and D, table 1).

If the physiological effect of the toxic agent upon the starfish egg is proportional to the product of concentration into time of exposure (as is the case, e.g., with the action of fatty acids on these eggs), a convenient measure of toxicity, in the case of any solution, is the reciprocal (3) of the time required to produce a given toxic effect (e.g., to prevent the development of surface swimmers, or to kill a certain proportion of eggs in a given time).

Judged by these criteria, solution D of table 1 is about twice as toxic as solution C, about three times as toxic as solution B, and about four times as toxic as solution A. In other words, in one hour at 20° the solution loses at least three-fourths of its initial toxicity.

A similar series of experiments was performed September 3, with three portions of a "mustard gas" solution made in the cold as above, and used in one-third dilution. The times of exposure to room temperature before using were respectively (A) twenty-four hours, (B) about one hour, and (C) one-half minute. As before, solution C was decidedly the most toxic, and solution A the least.

TABLE 4

MUSTARD SOLUTION AT ROOM TEMPERATURE	NUMBER OF MINUTES EGGS EXPOSED					
	1	2	4	8	16	32
(A) 24 hours	N	N	N	N	N	I
(B) 1 hour	N	N	I	I	I	D
(C) ½ minute	I	I	D	D	D	D

N = normal; I = injured; D = dead.

A detailed description of these experiments is unnecessary. The general results, which were closely similar to those recorded in table 2, have been tabulated as accurately as possible in table 4. In this experiment, in which the fresh saturated solution had undergone less decomposition than in some of the previous cases, the contrasts are even more striking, a one to two minute exposure to the fresh solution producing as great an effect as a four to sixteen minute exposure to the one hour old solution, or a thirty-two minute or longer exposure to a solution twenty-four hours old.

Similar experiments on September 2 and September 4 give results of the same general nature. In all cases the toxicity of the solution declined rapidly on standing at room temperature. This decline in toxicity, as stated above, is attributable to the hydrolysis of the mustard gas.

RELATION BETWEEN TOXIC ACTION AND HYDROLYSIS OF "MUSTARD GAS" SOLUTIONS

In a special experiment in which an ice cold distilled water saturated solution of "mustard gas" was prepared side by side with an ice cold saturated sea water solution, and no time was allowed to elapse between the preparation of the solutions and their utilization, the rate of hydrolysis in the distilled water was found to be such that half the "mustard" was decomposed in ten to twelve minutes, 75 per cent in twenty-three to twenty-five minutes, and 92.5 per cent in fifty minutes. A comparison, at the same time and under exactly similar conditions, of the decline in toxicity of the saturated sea water solution, at the same temperature of 21°, shows a striking correspondence between hydrolysis and toxicity (see fig. 2, p. 24).

While it is obvious that the crude methods of comparison available do not give an absolutely accurate index of toxicity, there is a fairly constant relation between the proportion of mustard remaining undecomposed in the solution, and its toxicity. This point is very well borne out by the titrations of solutions employed in the various experiments described above. In the experiment depicted in figure 2, p. 24, in which the fall in toxicity was rapid, the solution employed exhibited at the start an acidity equivalent to only 5 per cent of the total mustard contained. The solutions employed in the experiments of September 3, depicted in table 4, p. 22, showed the following comparative acidities when 20 cc. of solution was titrated with an $\frac{N}{14}$ NaOH solution, using di-brom-cresol-sulphone-phthalein as indicator: A = 0.85 cc.; B = 0.45 cc., and C = 0.25 cc.

The fact that the mustard was already partially decomposed in solution C, explains the fact that the difference in toxicity between the fresh solution and the solution one hour old was not so great in this experiment as in that depicted in figure 2; but, in the experiments described in tables 2 and 3, the effect of keeping the saturated solution for a prolonged period in the ice box is very marked. The titration results for 20 cc. are as follows: Table 2, solution A = 0.9 cc.; solution B = 0.85 cc.; solution

C = 0.85 cc.; solution D = 0.45 cc.—Table 3, solution A = 1.0 cc.; solution B = 0.95 cc.; solution C = 0.8 cc., and solution D = 0.75 cc. While these titrations of the solutions employed in the experiments described in tables 2, 3 and 4, carried out in sea water, are necessarily somewhat rough as compared with those depicted in figure 2, it is quite obvious that the diminution in toxicity exhibited by the solutions in table 2, and the still greater

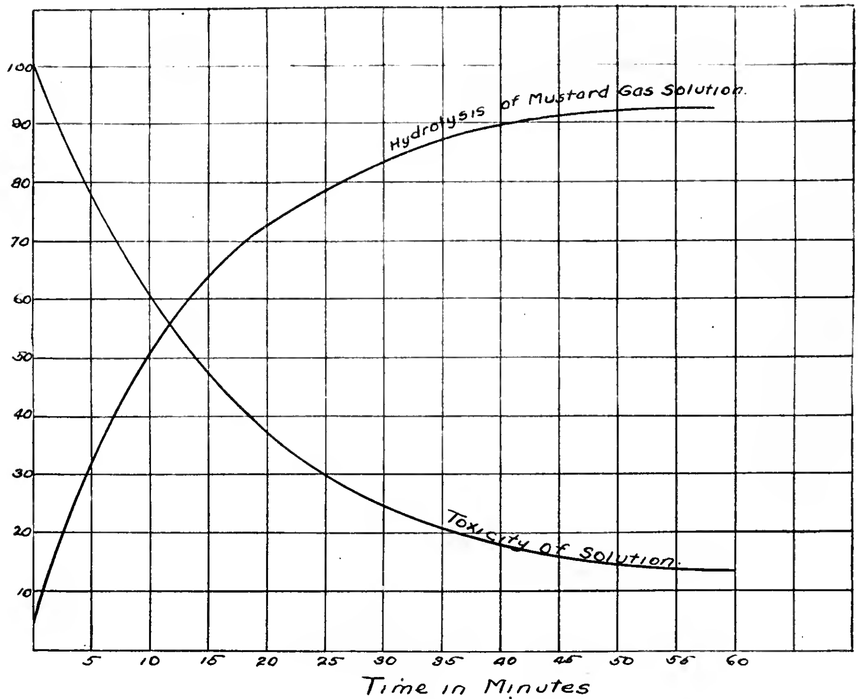

Fig. 2. Comparison of Rate of Hydrolysis of Saturated "Mustard Gas" Solution at 21°C., with Diminution in Toxicity

diminution shown in tables 3 and 4, are to be explained in great measure as attributable to hydrolysis which had taken place in the ice cold solution prior to the commencement of the experiment.

When first prepared, the saturated ice cold sea water solution has a slightly alkaline reaction like sea water, but the acid reaction develops as already described, slowly in the cold and more

rapidly at high temperature. The acidity developed from an ice cold saturated solution is, however, never sufficient to exert any disturbing effect on the developing eggs when diluted with three or four parts of normal sea water. Experiments in which the hydrochloric acid was exactly neutralized prior to admixture with additional sea water, showed that this was the case. From experiments with mustard solutions which had been allowed to stand for several weeks and were then neutralized, the di-hydroxy-ethyl-sulphide formed as the other product of hydrolysis, appears to be absolutely non-toxic, a conclusion recently reached by Marshall, who found this substance in the urine of animals poisoned with mustard gas, and also injected it in considerable quantities in normal animals, which remained unaffected.

While solutions which have stood at room temperature for a prolonged period are comparatively non-toxic, they are still capable of producing considerable irregularities in the development of marine eggs, indicating the presence of traces of undecomposed "mustard."

At an earlier stage in this report attention has been drawn to the apparent lag in loss of toxicity of the "mustard" solution as compared with the curve of hydrolysis. This lag is already recognizable in the experiments depicted in figure 2, page 24, after a period of only one hour; but even after three or four days the traces of "mustard" still remaining in the water appear to be strongly adsorbed by eggs introduced into the water. The fact that this toxic effect persists for so long a period is a strong argument in favor of the view that protoplasm exercises a selective affinity for "mustard" and removes even traces of this substance from solution.

To cite examples of experiments of this type, on August 26 fertilized eggs were exposed for thirty minutes to an undiluted solution of saturated sea water which had stood at room temperature for four weeks. This solution, which was filtered free from suspended particles, was neutralized with NaOH before adding the eggs. The majority of the eggs thus treated formed blastulae and gastrulae, some of which swam at the surface of the water. There was, however, some slight toxic action, shown in irregu-

larities of form, cilia less active than normal, etc. A similar experiment performed the same day with another mustard gas solution, which had stood at room temperature for more than two weeks, gave a similar result, but the toxicity was greater than in the case of the previous solution. The majority of eggs formed blastulae after an exposure of twelve minutes to the neutralized solution. A solution which had stood for only three days was found to be much more toxic. Less than 50 per cent of eggs formed blastulae after an exposure of only nine minutes to the neutralized solution.

Numerous confirmatory experiments gave the same result. Although the concentration of the undecomposed "mustard gas" in these solutions is so slight as to be scarcely recognizable by chemical means, it may have considerable effect with prolonged exposure of the eggs, presumably because of the progressive accumulation of the highly organo-soluble mustard in constituents of the cell protoplasm. There is a marked difference in toxicity between neutralized and un-neutralized saturated solutions of "mustard gas" in sea water, the former being less toxic, indicating that if free acid is present a portion of the toxic effect is due to this acid; but a careful comparison of the results leaves no doubt whatever that the external toxic effect of HCl is vastly less than its effect when applied internally through the agency of undecomposed "mustard."

Experiments with Arbacia eggs (August 15 and 17) showed that neutralization of partially decomposed saturated and half saturated solutions of "mustard" in sea water removed a small part but not all of the toxic action resulting from brief exposures of thirty seconds, one minute and two minutes, respectively. While a considerable proportion of eggs formed larvae in all cases, those developing from eggs exposed to the acid solution were fewer and less active (a smaller proportion came to the surface, and were less normal in appearance than those from the neutralized solution).

LATENCY IN THE ACTION OF MUSTARD GAS

The prolonged latency so characteristic of poisoning by this compound is shown also in starfish eggs in a typical manner. Eggs exposed for a brief period to moderately toxic solutions (within half an hour after fertilization) show at first little or no change in appearance and activity, and the first two or three cleavages may be normal in rate and regularity. Retardation and irregularity make their appearance by degrees, and by four or five hours after fertilization a distinct difference from normal eggs is usually apparent in the smaller number and less regular arrangement of the cleavage cells. The later development of such lightly poisoned eggs is more or less abnormal; the blastulae are typically less regular in form, thicker-walled, show less active ciliary movement, and undergo gastrulation (if at all) considerably later than normal eggs. Usually death, followed by granular disintegration of the cell protoplasm, occurs before the gastrula or later larval stages are reached. This description relates to eggs which are not too severely poisoned; in cases of severe poisoning cleavage is entirely prevented (cf. tables 2 and 3), or is from the first abnormal, and the eggs break down and disingrate without development.

The following description (table 5) of a series of experiments with eggs exposed for brief periods to a half-saturated solution of "mustard gas" (made six hours previously and kept at room temperature) will illustrate this point. The condition of the eggs about five hours after placing in the solution is described, and also the condition after about twenty hours. It will be noted that very brief exposures (one-quarter and one-half minute) greatly impair the later development of the eggs, although many reach the blastula stage; but the differences between the effects of unequal exposures (between one-quarter and one minute) are slight at five hours and become marked only later. Early cleavage is retarded (compare control), but to almost the same degree in the first four numbers of the series. Later the differences become much more pronounced.

TABLE 5

August 5. Eggs were fertilized at 3.05 p.m., and placed in one-half saturated solution of "mustard gas" at 3.45 p.m. Portions were returned to normal sea-water after the intervals cited.

NUMBER OF EXPERIMENT	TIME OF EXPOSURE	RESULT (CONDITION 5 HOURS AND 20 HOURS AFTER RETURN TO SEA-WATER)
	minutes	
1	$\frac{1}{4}$	*5 hours.* Cleavage stages are mostly regular 8- and 16-cell with some 32-cell.
		20 hours. Most eggs have formed blastulae; majority small, thick-walled or irregular; none gastrulated; ciliary movement slow; about 20 per cent of eggs disintegrated inside membranes.
2	$\frac{1}{2}$	*5 hours.* Cleavage is similar to experiment 1 or slightly less advanced.
		20 hours. Blastulae are smaller and feebler than in experiment 1; a larger proportion of disintegrated eggs: about 50 per cent.
3	$\frac{3}{4}$	*5 hours.* Cleavage is like experiments 1 and 2 on whole, mostly 8 to 16 cells, but irregularities are more frequent.
		20 hours. Great majority of eggs (about 70 to 80 per cent) are more or less disintegrated inside the fertilization-membranes; about 20 per cent of free blastulae, small and abnormal.
4	1	*5 hours.* Cleavage is very similar to 3; cleavage groups are somewhat less advanced and less regular than in experiments 1 and 2.
		20 hours. Disintegrated eggs are more numerous than in 3; about 10 per cent normal free blastulae.
5	$1\frac{1}{2}$	*5 hours.* Most eggs are in irregular 8- to 16-cell stages.
		20 hours. Almost all eggs are disintegrated inside membranes; a few partial blastulae remain alive.
6	2	*5 hours.* Cleavage is less advanced than in experiment 5, about 50 per cent are in irregular 4- to 8-cell stages; remainder about 12- to 16-cells; blastomeres are more irregular in size than in preceding experiments.
		20 hours. Almost all eggs are completely disintegrated. About 5 per cent have formed feeble partial blastulae.
7	$2\frac{1}{2}$	Little difference from experiment 6.
8	3	*5 hours.* Prevention and retardation of cleavage are more marked. About 20 per cent are uncleaved; remainder are mostly groups of 2, 3, and 4 round cells.
		20 hours. All are dead and disintegrated inside membranes.

Control of untreated eggs: *5 hours.* Typical regular cleavage of about 128 cells.
20 hours. Almost all eggs have formed active regular gastrulae. Development is normal.

The initial effect of the "mustard gas" (for the first few hours) shows little difference in the first three or four members of the series. The later development shows that the quantity of poison taken up by the eggs is greater with the longer exposure. The poison asserts its action gradually, just as if its effect were due to the progressive liberation of some destructive compound within the protoplasm. Other similar series of experiments have given the same general result.

If the toxic action of "mustard gas" is in fact due to liberation of hydrochloric acid within the cell, it ought to be possible to diminish or prevent this action by the use of basic substances which readily penetrate protoplasm. The possibilities of this form of treatment should not be exaggerated, since bases like ammonia have in themselves a markedly toxic action, and any excess of such compounds would in itself be injurious. We have, however, carried out a large number of experiments in which eggs poisoned by "mustard gas" were subsequently treated for varying periods with weak solutions of ammonia in sea water. Experiments have also been made with a lipoid-soluble organic base, aniline. The general result has been that ammonia has undoubtedly a certain antagonistic or anti-toxic effect when employed as an after-treating agent in low concentration. The effect, however, is not great, and may readily be masked by the toxic effects of the ammonia itself. Previous treatment of the eggs with aniline solution also has a certain protective effect, but this may be attributable to the anaesthetic action of the compound, rather than to its basicity as such. Experiments with Arenicola larvae (to be described below) indicate that this is the case; here previous exposure to aniline solutions has a distinct protective action, while after-treatment is ineffective, and other anaesthetics (alcohols) give some protection, although in our experiments this was less marked than with aniline (see below, p. 36).

EXPERIMENTS ON AFTER-TREATMENT OF POISONED STARFISH EGGS WITH WEAK SOLUTIONS OF AMMONIA

Seven series of experiments were performed at different times in which fertilized eggs, poisoned by exposure to mustard gas so-

lutions for varying periods, were afterwards treated with weak solutions of ammonia in sea water, of concentrations ranging from n/500 to n/3000, for periods ranging from one-half hour to four hours. In general only the weaker solutions of ammonia proved effective, viz., n/2000 and n/3000 (in one case n/1000 gave slight improvement), and exposures of more than three hours were unfavorable. Solutions of NH_3 in sea water are themselves strongly toxic to normal eggs. Two hours' exposure to n/250 NH_3 kills all eggs; a similar exposure to n/500 NH_3 has a distinct and to n/1000 NH_3 a slight injurious action. Four hours in n/1000 NH_3 is markedly injurious while the same exposure to n/2000 NH_3 has little effect. The favorable effect of after-exposure to n/2000 NH_3 is distinct if the exposure is not too prolonged and if the previous degree of "mustard gas" poisoning is slight.

The following table summarizes the results of a series of experiments performed on August 26. A mildly toxic solution of mustard gas was used and the fertilized eggs were exposed to the neutralized solution for two periods, (A) seven minutes and (B) twelve minutes. Eggs from each poisoned lot (A and B) were transferred directly from the "mustard" solution to sea water and to n/2000 NH_3 solution in sea water, respectively; from the latter medium portions were returned to normal sea water after the four intervals, (a) 30 minutes, (b) one hour, (c) one and one-half hours and (d) two and one-half hours.

This series is fairly representative of the experiments with ammonia. Exposures of not more than three hours to n/2000 NH_3 effected a distinct though slight improvement in every series of experiments. A similar series with Arbacia eggs also gave this result. While the anti-toxic effect was never strongly marked, it seemed clear that the action of the absorbed mustard gas within the cell had been somewhat delayed, presumably by neutralization of the acid freed during the period of immersion in the ammonia solution. With too long exposures or too concentrated solutions of NH_3 the toxic action of the latter compound asserts itself, and no antitoxic action is seen. These experiments again favor the theory that the toxic action of "mustard gas" depends on intracellular liberation of acid.

A series of experiments in which the eggs were treated with a one-eighth saturated solution of aniline in the sea water (the anaesthetizing concentration for Arenicola larvae), for about

TABLE 6

Series A. Starfish eggs fertilized at 10.40 were exposed to the neutralized "mustard gas" solution for 7 minutes (11.12 to 11.19). Part of the eggs (1) were then returned to normal sea-water, another part (2) to n/2000 NH_3 for the periods indicated. The condition of the eggs next morning were as follows:

1	*Returned directly to seawater.* Most eggs form irregular blastulae and gastrulae with characteristically crinkled walls.
2	*In n/2000 NH_3 as follows:*
	(a) *30 minutes* (11.19 to 11.49). The proportion of larvae is not greater than in 1, but their condition is better and the proportion of normal-looking symmetrical gastrulae is larger.
	(b) *1 hour* (11 19 to 12.19). Here there is little difference from control.
	(c) *1½ hours* (11.19 to 12.49). Numerous irregular blastulae; little difference from control.
	(d) *2½ hours* (11.19 to 1.50) Not much difference from control, but on the whole the larvae are more regular

Series B Exposed to the same solution for 12 minutes (11.12 to 11.24).

1	*Direct to seawater* Most eggs form irregular or undersized blastulae with crinkled walls.
2	*In n/2000 NH_3 as follows:*
	(a) *30 minutes.* (11.24 to 11.54). Little difference from control.
	(b) *1 hour.* (11.24 to 12.24). Distinct improvement; blastulae are more regular and a fair proportion of nearly symmetrical gastrulae are present.
	(c) *1½ hours* (11.24 to 12.54). Again the blastulae are more regular and less crinkled and the gastrulae more symmetrical than in the control.
	(d) *2½ hours* Larvae show distinct improvement as in 2c.

twenty minutes previously to placing in the "mustard gas" solutions, also gave some evidence of protection. The aniline-treated eggs cleaved more regularly and gave a somewhat higher proportion of regular larvae than the eggs exposed to "mustard

gas" for the same period without this treatment. A similar protective effect was observed with Arenicola larvae (see below) and its significance is somewhat uncertain. Probably a certain stabilizing action, of the same nature as that frequently observed with anaesthetics, is responsible for the effect, although the basicity of the compound may play some part. The experiments with Arenicola larvae (to be described below) give more definite evidence on this point. The use of lipoid-soluble basic compounds as a possible antidote to "mustard"-poisoning has been suggested as a promising subject for investigation. The influence of other lipoid-soluble compounds, including anaesthetics, should also be investigated.

EXPERIMENTS WITH AMYL ALCOHOL

We have also studied with starfish eggs the influence of amyl alcohol on the toxic action of mustard gas, proceeding on the supposition that the presence of a second highly lipoid-soluble and surface-active compound might influence the distribution of the "mustard," and in this manner either intensify or diminish its action. The results of this study—which will be reported briefly—were in certain respects definite and suggestive, but seem difficult of interpretation.

On August 10 fertilized starfish eggs were exposed to a freshly prepared mustard gas solution, using the four concentrations one-half, one-fourth, one-eighth and one-sixteenth saturated; two times of exposure, one-half minute and three minutes, were employed with each solution. One set of solutions (A) was used pure, the other (B) contained amyl alcohol as follows: (1) one-half saturated "mustard gas" solution containing 1 volume per cent amyl alcohol; (2) one-fourth saturated plus 0.5 volume per cent alcohol; (3) one-eighth saturated plus 0.25 volume per cent alcohol; (4) one-sixteenth saturated plus 0.13 volume per cent alcohol. Exposures to the one-half saturated mustard, pure and amyl-containing, for three minutes killed all eggs: with the one-half minute exposure a minority of eggs formed blastulae, which were more numerous in the amyl-treated lot. With the

other three pairs of solutions the presence of the amyl alcohol had in every case a protective effect; the larvae were more numerous and better developed, and showed more active ciliary movement.

Two other similar series of experiments on August 7 and 8 also showed distinct protection in the alcohol-containing series of solutions. In two series of similar experiments with Arbacia eggs amyl alcohol showed a less favorable influence, although some protection was apparent.

These results are in all probability to be attributed to a certain stabilizing action of the amyl-alcohol on the cell-structures, such as is shown in the protective influence of this and other anaesthetic compounds on marine eggs and larvae exposed to pure salt solutions (4).

Some effect may perhaps be attributed to a partial displacement of the toxic compound from the adsorbing surfaces of the protoplasmic system. Pharmacological interference-effects of this general kind should be further investigated.

EXPERIMENTS ON OTHER MARINE ORGANISMS

Experiments were also performed to demonstrate the toxic action of weak solutions of mustard gas on a variety of marine organisms. These included ciliated larvae (of sea urchin, starfish, the annelids Nereis and Arenicola), freshly hatched squid larvae, and freshly hatched fish (Fundulus). All showed characteristic toxic effects. Nothing especially noteworthy was observed in these experiments except the prolonged latency of the toxic action. The organisms swam for several hours in a partially decomposed saturated solution of "mustard gas" in sea water, and only gradually ceased movement and disintegrated. Similar effects were observed with spermatozoa.

More systematic experiments were carried out with the free-swimming larva of the annelid Arenicola. This organism has been much used in physiological experimentation at Woods Hole, largely because of the ease with which it may be obtained in large quantity, and the definiteness and constancy of its behavior and

other properties. It is a small maggot-like animal, about one-third of a millimeter in length, swimming actively by two ciliated rings and exhibiting a strong positive phototaxis. Large masses of these organisms are readily obtained by hatching from the egg-strings, which are abundant in the sand-flats of certain localities near Woods Hole. The larvae on hatching collect rapidly on the side of the dish directed toward the source of light. A peculiarity of this positive phototaxis is that it may readily be reversed, or rendered negative, by the addition of various chemical substances to the sea water, especially lipoid-soluble compounds. The organism may thus be used as a convenient indicator of the relative intensity of action of such compounds.

REVERSAL OF PHOTOTAXIS OF ARENICOLA LARVAE, AND ANILINE PROTECTIVE EFFECT

The effect of "mustard gas" in reversing phototaxis is interesting, since the same effect is produced by acid, as well as by lipoid-solvent compounds of various kinds (alcohols, esters, hydrocarbons, ethers, etc.) in appropriate concentrations (5). If normal positively phototactic larvae are transferred for a few minutes from normal sea water to sea water containing a reversing compound, the organisms, on return to sea water, collect on the side of the dish *away from* the source of light instead of (as formerly) on the side toward the light. This negative response is, as a rule, temporary and the normal positive reaction returns within one or two hours or less. Weak solutions of "mustard gas" in sea water produce this reversal in a typical manner; but what is significant of the mode of action of this compound is that the return of the normal positive reaction in normal sea water is greatly retarded as compared with that following reversal by acid alone or by chemically indifferent organic compounds like the anaesthetics. This persistency of the negative reaction is an indication that the compound continues to exercise its effect for a considerable time after the organism is returned to sea water, and supports the view that a continued liberation of acid from the lipoid-bound "mustard gas" within

the protoplasm is the cause of the special peculiarities of poisoning by this compound.

The following experiments illustrate these effects. On August 3 Arenicola larvae were placed in solutions of "mustard gas" (prepared by shaking with the oil on July 31 and since then standing in contact with the oil); four dilutions were used; saturated, one-half, one-fourth and one-eighth saturated; the larvae were returned to normal sea water after two exposures, ten minutes and two hours respectively. For comparison larvae were also exposed to slightly acid sea water (100 cc. sea water *plus* 2.5 cc. n/14 HCl) for similar periods; this solution has approximately the same effect in reversing phototaxis as the one-fourth saturated "mustard" solution. The partially decomposed saturated and half-saturated "mustard" solutions killed all larvae in ten minutes. In the one-fourth saturated solution the larvae remained living after two hours exposure; it was further noted that during immersion in this solution they exhibited the muscular immobility or stiffness characteristic of anaesthesia in this organism; this effect is interesting, since it indicates that "mustard gas," apart from its characteristic toxicity, has anaesthetic properties like other lipoid-solvent compounds.

On return to sea water from the one-fourth and one-eighth saturated solutions all larvae became negatively phototactic and remained in this condition for some hours. The persistence of the negative reaction was greater with the two-hour than with the ten minute exposure; after eighteen hours in sea water about half the larvae of the two hour lot still showed a negative response. The larvae reversed by a similar exposure to acid sea water were all positive by this time. A repetition of this experiment gave a similar result; the phenomena of reversal are similar with mustard-treated and acid-treated larvae, but the persistence of the effect is far greater in the former case.

Experiments were also performed in which larvae were exposed to one-half saturated partially decomposed "mustard" solutions for briefer periods, viz., $\frac{1}{4}$ minute, $\frac{1}{2}$ minute, 1, 2, 3, and 4 minutes, and also, for comparison, to acid sea water (5 cc. n/14 HCl *plus* 100 cc. sea water) for the same periods. Exposures of fifteen

seconds to both the "mustard"-containing and the acid sea water caused reversal of phototaxis in the majority of larvae. The same effect was produced by exposures up to one minute; two minutes' exposure to either solution was destructive. The reversing action was similar in both solutions, but the return of normal positive phototaxis was much more rapid in the acid-treated larvae; thus after one minute in either solution most larvae showed a negative reaction in sea water two hours later; after six hours in sea water the great majority of "mustard"-treated larvae were still negative (although some were positive), while two-thirds of the acid-treated were again positive.

Aniline was found to have a distinct protective influence against the destructive effect of "mustard gas" on Arenicola larvae. One-fourth saturated solutions of aniline in sea water induce typical anaesthesia (muscular immobility, cessation of phototactic swarming) in these organisms; on replacing in sea water the normal reactions return promptly. Larvae which had been kept in one-fourth saturated aniline solutions for one hour were transferred directly to a one-half saturated "mustard gas" solution and exposed for periods of 1, $1\frac{1}{2}$ and 2 minutes. For comparison normal larvae received similar exposures at the same time. The destructive effect of the "mustard" was distinctly less in the aniline-treated larvae; one minute's exposure destroyed permanently all phototactic response in the normal larvae, while next day many of the aniline-treated larvae showed active swimming and phototaxis. Exposures of one and one-half and two minutes destroyed the cilia almost completely in normal larvae; in the aniline-treated larvae ciliary movement remained next day in a large proportion; after two minutes exposure all normal larvae were found dead and partly disintegrated next day, while of the aniline-treated larvae a fair proportion survived.

This protective effect is probably to be referred in large part to the anaesthetic action of the aniline, which, like anaesthetics in general (6), renders this organism more resistant to subsequently acting injurious conditions; but a portion of the effect seems to be due to the basicity of the compound. This is indicated by the following experiments. Larvae were exposed for

ten minutes to anaesthetizing solutions of a series of alcohols in seawater, as follows: 5 volumes per cent ethyl; 2 volumes per cent n-propyl; 0.8 volume per cent n-butyl; 0.3 volume per cent i-amyl; they were then transferred directly to a strongly toxic "mustard gas" solution (which killed all control animals with two minutes' exposure); exposures of 1, 2, and 4 minutes were used. All of the alcohol-treated animals withstood the one minute exposure better than the control; the cilia remained largely intact and muscular contractility was impaired to a less degree. The effect, however, was less striking than with aniline, indicating that this latter compound possesses some special favorability, probably due (as suggested) to its basic property.

Attempts were also made to counteract the toxic action of "mustard gas" by treating larvae, *after* the exposure to the poison, with one-fourth saturated aniline solutions; but no favorable result was obtained. The protective effect of this treatment is evidently much greater if it precedes than if it succeeds application of the toxic agent. A certain resistance to the subsequently acting toxic agent is thus imparted.

EXPERIMENTS WITH FISH

The experiments with fish (Fundulus) showed again the long latent period of action and the necessity that the compound should be absorbed by the living cells while it is still in the intact non-hydrolyzed state.

The following experiment (September 10) will illustrate: Four active and healthy adult Fundulus were placed in a one-fourth dilution of each of the following solutions: (A) solution of "mustard gas" prepared in the cold and filtered and then kept for five days at room temperature before using; (B) a similar solution made the day before and kept at room temperature for twenty-four hours befor using; (C) the same solution as (B), but kept at 0° and brought to 20° one-half hour before using; (D) the same solution as B and C kept at 0° until immediately before using. Each solution was of one-fourth saturated concentration.

The results were as follows: Solutions A and B proved almost non-toxic, three out of the four fish remaining alive and apparently normal after six days in the solutions. In solution C all four fish were alive three hours after placing in the solution, but were crowded at the surface of the aquarium and breathing heavily; after eighteen hours three were dead, and the other died within twenty-six hours. In the case of solution D, three hours after placing the fish in the solution, two were dead, one was dying, and the third was at the surface breathing heavily; next morning (at eighteen hours) all were dead and in an advanced state of *rigor mortis.*

The rapid decline in toxicity on standing at room temperature is again well shown in this experiment. Old decomposed solutions of "mustard gas" in sea water have little effect on Fundulus, both adults and freshly hatched fish remaining alive for days and exhibiting quite normal behavior.

INFLUENCE OF TEMPERATURE ON TOXIC EFFECT OF "MUSTARD GAS"

In two series of experiments the relative rates of action of a strongly toxic "mustard" solution at two temperatures, about 10° apart, were determined. The rate of toxic action was found to exhibit a high temperature-coefficient ($Q_{10} = 3$–4). Fertilized starfish eggs were exposed for periods of 1, 2, 4, and 8 minutes to two portions of the same solution (one-half saturated "mustard gas" prepared two days previously), one being kept at 9 to 10°, the other at 21° during the period of exposure. An exposure of two to four minutes at 21° was found to injure the eggs so that none of the four-minute lot and only a few of the two-minute lot formed surface swimmers. At 9 to 10° the same degree of injury required an exposure of eight minutes; eggs exposed for four minutes at this temperature formed numerous surface gastrulae. An exposure of eight minutes at 21° killed all the eggs; after the same exposure at 9 to 10° the great majority of eggs formed blastulae. Four minutes exposure at 21° had somewhat more effect and two minutes at 21° somewhat less effect than eight minutes exposure at 9 to 10°.

The above summarizes the results of one series of experiments; in the other series a similar difference in the rate of action at the two temperatures (10° and 21°), was found. In this case the solution used was more toxic than in the first series and the destructive action was pronounced even at 10°.

This difference in rate of action at the two temperatures is similar to that found by R. S. Lillie in the rate of action of butyric acid on these eggs (7). This fact again indicates that the destructive action is due to the acid freed from the compound. The rate at which this acid produces its physiological effect is a function of temperature; the temperature-coefficient is similar to that of the majority of chemical reactions at or near these temperatures. Other factors, including the rate of hydrolysis of the "mustard gas," are also influenced by temperature, so that the total effect of change of temperature is complex. It is clear, however, that the destructive action may be greatly retarded by cold.

INTRAVITAM STAINING

To ascertain in a more direct manner the effect of "mustard gas" and of its decomposition products on the cell, two methods were used, first, a comparison of the intra vitam staining properties of treated and non-treated cells and, second, the effect of the injection of solutions directly into the interior of the cell.

Staining with neutral red. By the use of intra vitam staining strong evidence was afforded that free acid is liberated within the cell. The intravitam stain used was neutral red. Fertilized starfish eggs were placed in a solution of mustard gas in sea water (1 part of a concentrated sea water solution to 3 parts sea water) for varying lengths of time, i.e., from one to six or eight minutes. The treatment was only enough to cause subsequent abnormal development. After being washed in fresh sea water the treated eggs were placed in a dilute solution of neutral red in sea water (one drop of a carefully neutralized aqueous solution in 100 cc. sea water). As a control normal eggs were treated with a neutral red solution of the same strength. The control and the gassed eggs were compared from time to time and the differences

in staining properties noted. The rapidity and intensity with which the eggs stain with the neutral red depends on the length of time the eggs are in neutral red solution and on the concentration of the stain. No difference in the rapidity of staining was noted in the control and the gassed eggs. The eggs absorb the stain from enormous dilutions and progressively intensify in color as long as the dye is present in the surrounding water.

TABLE 7

Table showing two examples of the experiments when the eggs were treated with the "mustard gas" solution and later stained with neutral red

Exp. 1	Eggs fertilized at 11.10 a.m.				
	Gassed at 11.39 a.m.	for 2 min.	for 3 min.	for 4 min.	Untreated eggs
	Stained with Neutral red at 12.09 p.m.				
	Examined at 12.13 p.m.	Slightly tinged	Slightly tinged	Slightly tinged	Slightly tinged
	Examined at 12 17 p.m.	Heavily tinged	Heavily tinged	Very heavily tinged	Slightly tinged
Exp. 2	Eggs fertilized at 12.40 p.m.				
	Gassed at 3.00 p.m.	for 1½ min.	for 6 min.	for 8 min.	Untreated eggs
	Stained with Neutral red at 3.50 p.m.				
	Examined at 4.10 p m.		All tinged on periphery		
	Examined at 4.20 p.m.		Heavily tinged throughout	Heavily tinged throughout	Slightly tinged throughout
	Examined at 5.00 p.m.		Heavily tinged throughout	Very heavily tinged throughout	Slightly tinged throughout
	Examined at 7.00 p.m.		Heavily tinged throughout	Very heavily tinged throughout	Slightly tinged throughout

At first the periphery alone of the eggs is colored. The color then passes into the interior of the cells staining the cytoplasm diffusely. Gradually, however, the color is taken up by certain cytoplasmic granules which collect about the division centers of the cells. Within an hour or so after treatment the cytoplasmic granules in the gassed eggs are distinctly more intensely stained than those in the untreated "control" eggs. It was impossible to detect a difference in brightness of the rose red colored granules, but there is no doubt that the stained granules in the gassed eggs are deeper in color. The stained granules stand out in very sharp contrast to the surrounding cytoplasm, and they appear to be more numerous in the gassed eggs.

The experiment was repeated a number of times and always with the same result, i.e., the cytoplasmic granules in the gassed egg became progressively more intensely stained so that within an hour or so after treatment the greater intensity in color as compared with the control was easily recognizable (see table 7).

In contrast to the definite results obtained when the treated eggs are stained subsequently with neutral red are the results when the eggs are first stained with neutral red and later treated with the "mustard gas" solution. In the latter case no differences could be observed between the gassed and the control eggs, indeed, in several cases the normal eggs appeared to be more heavily stained. Neutral red is somewhat toxic, and this fact may obscure the results when the eggs are stained before being treated.

INJECTION OF UNDECOMPOSED AND DECOMPOSED "MUSTARD GAS" SOLUTIONS INTO THE CELL

Barber's pipette holder was used to test the effect of the "mustard gas," and its decomposition products on the cell interior. The apparatus consists of a mechanism of screws to which a glass pipette or a needle can be clamped (8). The screws are so arranged that the pipette or needle may be moved in any of three directions at right angles to one another. Even under the oil immersion objective a fairly steady and even movement is pos-

sible. The apparatus is attached to the side of the microscope, and the pipette is so adjusted as to project horizontally into a moist chamber on the stage of the microscope. The tip of the pipette reaches into a hanging drop suspended from the roof of the moist chamber. The roof of the moist chamber consists of a large coverslip, and in the hanging drop are the cells to be operated upon. Hard glass tubing with an outside bore of about 2 mm. is drawn out into a capillary, and the tip of the capillary is drawn again and allowed to fuse to a point in a minute acetylene flame. When properly made the bore of the tube extends almost to the very point of the needle. The needle is then placed in the apparatus and the point brought into a hanging drop of the fluid to be injected, and the screws are turned until the point is brought under observation in the field of the objective. The tip of the needle is then raised until it strikes the under surface of the coverslip. This snaps off the tip, converting the needle into a pipette, upon which fluid is sucked into the pipette. The diameter of the aperture of a successfully made micropipette averages a little over 0.001 mm. (1 micron).

Expansion of mercury by heat is used as a driving force for the injection. The glass tubing is filled with mercury and one end sealed before the other end has been converted into a pipette.

The flow of liquid from the micropipette is gradual enough not to cause mechanical injury to the cell. Protoplasm very readily forms precipitation- or coagulation-films, and if fluid be allowed to flow too rapidly into a cell a vacuole is formed which persists for a longer or shorter time. Aqueous solutions, when allowed to flow into a cell slowly and gradually, mix insensibly with the cytoplasm. If the fluid is injurious cytolysis sets in; if it is not immediately injurious the cytoplasm is slowly diluted and the cell swells in size.

Because of difficulties in the technique the operation has to be performed a large number of times before one can be satisfied with one's result. Delay after the puncture has been made often leads to the formation of a surface film around the puncture, so that the tip of the pipette comes to lie in a deep pit in the side of the egg instead of penetrating into the interior. In

such a case the injection fluid flows out of the pit back along the pipette without entering the cell.

The following are the results obtained with the microinjection method.

1. Eggs may be injected with distilled water, or with sea water, and no deleterious effects arise so long as the amount injected produces no appreciable swelling of the egg. The majority of such eggs develop normally at about the same rate as untreated eggs. This result has been obtained in a large number of experiments and may be considered fairly certain.

TABLE 8

SOLUTION EMPLOYED	EFFECT ON CELL EXTERIOR (SEA WATER)		EFFECT ON CELL INTERIOR	
	Immediate	After 4 hours	Immediate	After 4 hours
Water	Nil	Nil	Nil	Nil
Undecomposed "mustard"	Nil	Necrosis	Nil	Necrosis
Decomposed "mustard"	Nil	Very slight deformity	Necrosis	Necrosis
HCl equivalent to decomposed "mustard"	Nil	Very slight deformity	Necrosis	Necrosis

2. Eggs injected with a freshly made saturated aqueous solution of "mustard gas" show no immediate injurious effects. Their subsequent development, however, is always either very irregular or entirely inhibited.

3. Eggs injected with a saturated gas solution which has been allowed to stand at room temperature for several hours show an immediate destructive effect. The area immediately about the aperture of the pipette undergoes cytolysis which may extend throughout the entire cell. Frequently the cytolysis is prevented

from spreading through the cytoplasm by the formation of a surface film around the cytolyzed area. The surviving portion of the egg may then be fertilized, and a more or less irregular development results.

4. Eggs injected with an aqueous solution of hydrochloric acid of the same acid strength as the decomposed "mustard gas" solution exhibit an essentially similar effect, viz., a more or less extended cytolysis.

In table 8 a comparison has been made of the effects exerted by various solutions on the cell exterior and interior, observations being made both at once and after a period of four hours.

These experiments lend substantial support to the view that "mustard gas," in virtue of its lipoid solubility, penetrates the cell interior where it liberates hydrochloric acid.

SUMMARY

1. Developing eggs and larvae of starfish and sea urchins, larvae of Arenicola, Nereis, etc., and young and adult fish (Fundulus) have been used to study the nature of the toxic effects exerted by mustard gas on protoplasm.

2. In order to secure a true solution of "mustard gas" and to reduce hydrolysis to a minimum, saturated solutions in sea water and distilled water were prepared at a temperature of 0°C.

3. One of the most characteristic features of "mustard gas" poisoning is the latent period which elapses before retardation and deformation develop as a result of exposure to dilute solutions, and necrosis and death after exposure to concentrated solutions.

4. The toxic action, which may be measured by variations in length of exposure and concentration of solution required to produce given effects, is found to exhibit an increase of rate with increase in temperature in a manner comparable to that of a chemical reaction.

5. "Mustard" solutions gradually lose their toxicity on standing, and more rapidly at high than at low temperatures. A any given temperature the curve of loss of toxicity appears to corre-

spond very closely with the monomolecular curve of hydrolysis of mustard, indicating that undecomposed "mustard" is the toxic agent.

6. Di-hydroxy-ethyl-sulphide, one of the products of hydrolysis, is nontoxic, and the acid formed, if not neutralized by the buffer system of sea water, produces a less marked and somewhat different effect from that of undecomposed "mustard."

7. A lag in loss of toxicity, as compared with hydrolysis, after decomposition has proceeded for a considerable period, suggests a strong selective adsorptive capacity for "mustard gas" possessed by protoplasmic structures.

8. Attempts to counteract the effect of "mustard gas" by subsequent exposure of the developing eggs to weak solutions of ammonia, aniline, amyl alcohol, etc., have met with very limited success.

9. Arenicola larvae exhibit very persistent negative phototaxis after exposure to concentrations of "mustard" insufficient to cause death. This persistence is probably due to deferred production of acid within the organism.

10. "Mustard gas" appears under certain circumstances to exert an anaesthetic effect upon Arenicola larvae.

11. Intravitam staining affords some evidence of abnormally rapid production of acid within the protoplasm of poisoned eggs.

12. Injections of fresh aqueous solutions of "mustard gas," and of aqueous solutions which have undergone hydrolysis, into the interior of the eggs by means of a capillary pipette, show that undecomposed "mustard" when first injected exerts no greater effect than distilled water or salt solution, but after a latent period corresponding with that observed when mustard is applied externally, causes necrosis and death of the cell. The decomposed "mustard" solution when injected into the cell causes an immediate destructive effect corresponding with that produced by a solution of hydrochloric acid of the same strength, and far in excess of the effect obtained by application of the mineral acid to the exterior of the cell.

13. These and other experiments lend strong support to the theory advanced by Lynch, Smith and Marshall (1), that "mus-

tard gas" penetrates the cell on account of its organo-solubility, and within the cell undergoes hydrolysis with the liberation of nascent hydrochloric acid, which exerts the destructive effect.

14. The prolonged latent period may well be explained as attributable either to the high solubility of "mustard" in lipoids, retarding its passage into the water phase, or the formation of protoplasmic "mustard gas" molecular aggregates which undergo hydrolysis less rapidly than "mustard" in contact with water.

REFERENCES

(1) Marshall, E. K., Jr., Lynch, Vernon, and Smith, Homer W. On dichloroethylsulphide, the systemic effects and mechanism of action. Jour. of Pharmacol. and Exper. Therap., xii, 291, 1918.

(2) Smith, Homer W., Clowes, G. H. A., and Marshall, E. K. Jr. On dichloroethylsulphide, the mechanism of absorption by the skin. Jour. of Pharmacol. and Exper. Therap., xiii, 1, 1919.

(3) Lillie, R. S.: Jour. of Biol. Chem., xxiv, 233, 1916.

(4) Lillie, R. S.: Amer. Jour. of Physiol., xxix, 372, 1912; xxx, 1, 1912, and xxxi, 255, 1913.

(5) Kanda, S.: Biological Bulletin, xxxvi, 149, 1919.

(6) Lillie: Loc. cit.

(7) Lillie: Biological Bulletin, xxxii, 131, 1917.

(8) Chambers: Biological Bulletin, xxxiv, 121, 1918.

THE EFFECT OF PYRETICS AND ANTIPYRETICS ON CATALASE PRODUCTION

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There are a large number of substances (pyretics) tetrahydro-β-naphthylamin, caffein, atropin, cocaine, adrenalin, sodium chloride, etc., which when introduced into the body produce fever. There are also substances (antipyretics), quinin, acetanilid, phenacetin, etc., which decrease the temperature of the body if fever exists, but have little or no effect on normal temperature, while narcotics, such as chloroform and ether, lower temperature even in a normal animal. The attempts that have been made to explain the mode of action of pyretics in producing an increase and of antipyretics in producing a decrease in temperature have been made for the most part by studying the relation between heat formation and heat loss, it being evident that the temperature of the body depends upon the relation between heat production and heat elimination. For example, the ingestion of food increases heat production very greatly, and a cold bath heat elimination, yet neither of these produce practically any change in the temperature of the body owing to the accompanying increase in heat dissipation following the ingestion of food and heat production during a cold bath. Tetrahydro-β-naphthylamin on the other hand produces a rise in temperature by increasing heat production and decreasing heat loss, while antipyrine according to Stuhlinger (1) increases both heat production and heat loss, the fall of temperature produced by the drug being due to the disproportionate increase in heat dissipation. Gottlieb (2), however, holds that antipyrine lowers temperature solely by increasing heat radiation and that quinin acts by diminishing heat production. No attempt will be made in this

paper to give a review of the literature on the subject, or to discuss the different views held as to the mode of action of pyretics in increasing and antipyretics in decreasing temperature.

Of the two factors, heat production and dissipation, that are involved in bringing about a change in the temperature of the body, only heat production is dealt with in this paper. We (3) had already found that whatever increased oxidation in the body, the ingestion of food for example, produced an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme, and that whatever decreased oxidation, narcotics for example, produced a decrease in catalase by decreasing the output from the liver and by direct destruction of the enzyme. The present investigation was begun to determine if certain sybstances which are known to produce fever and at the same time increase oxidation when introduced into the body would stimulate the liver to an increased output of catalase, and what effect substances which decrease fever would have on catalase production. The names and amounts of the substances used will be given in the body of the paper. The animals used were dogs and rabbits. The catalase in the blood was determined by adding 0.5 cc. of blood to diluted hydrogen peroxide in a bottle at approximately 22°C., and as the oxygen gas was liberated it was conducted to an inverted, graduated vessel, previously filled with water. After the oxygen gas thus collected in ten minutes had been reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The material was shaken in a shaking machine at a fixed rate of one hundred and eighty double strokes per minute during the determination.

After exposing the jugular vein and opening the abdominal wall of a dog with the use of ether anesthesia, 0.8 gram per kilo of tetrahydro-β-naphthylamin in 200 cc. of water were introduced into the upper part of the small intestine. The catalase in the blood of the liver, jugular, and portal veins was determined before as well as at certain fixed intervals after the in-

troduction of the material. The results of the determinations are given in figure 1. The continuous line curves were constructed from data obtained from the blood of the liver, the discontinuous line curves from the blood of the portal vein, and the dotted line curves from the blood of the jugular. It may be

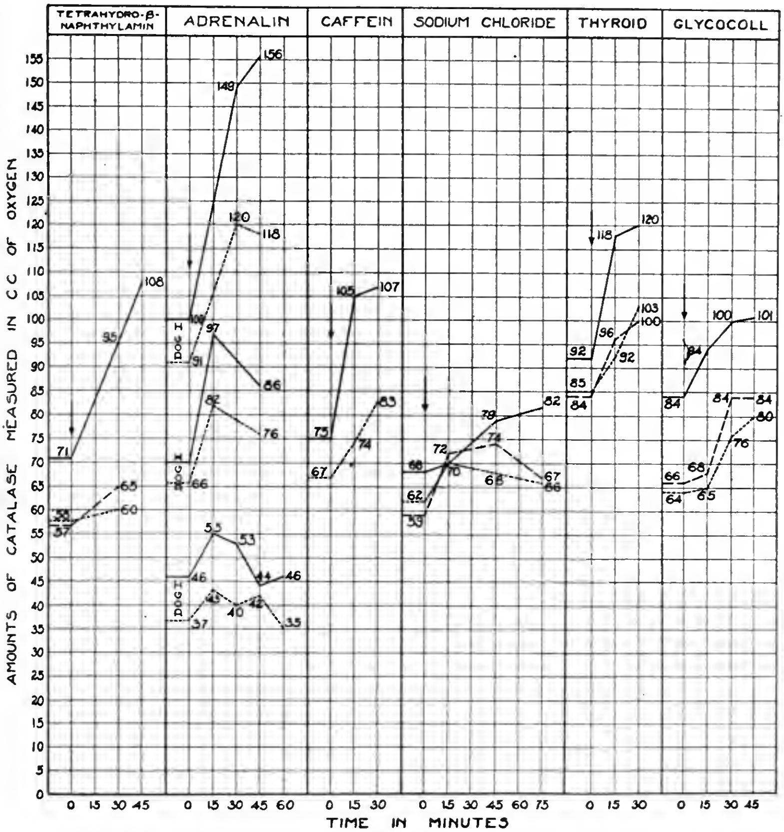

Fig. 1

seen that previous to the introduction of the material into the intestine 0.5 cc. of blood from the liver liberated 71 cc. of oxygen from hydrogen peroxide in ten minutes, and the blood from the jugular liberated 58 cc. and that from the portal 57 cc.; thirty minutes after the introduction of the material the blood of the liver liberated 95 cc. of oxygen and that of the jugular 60, and

of the portal 65; and forty-five minutes after the introduction of the material the blood of the liver liberated 108 cc. of oxygen. By comparing these figures it may be seen that tetrahydro-β-naphthylamin produced an increase in the catalase of the blood and that this increase was greater in the blood of the liver than in the blood of the portal and jugular veins. This is taken to mean that the tetrahydro-β-naphthylamin was stimulating the liver to an increased output of catalase. Similarly it may be seen that adrenalin, caffein, sodium chloride, desiccated thyroid, and the amino acid, glycocoll, produced an increase in the catalase of the blood due to the stimulation of the liver to an increased output of this enzyme. Three cubic centimeters of a 1:1000 solution of adrenalin chloride were introduced into the portal vein in dog III, figure 1; 5 cc. in dog II. In these two dogs, the solutions were injected as quickly as could conveniently be done. In dog I, 10 cc. of a 1:1000 adrenalin solution diluted to 50 cc. were injected at a rate of approximately 2 cc. per minute, requiring about thirty minutes for the injection. The sodium chloride, 10 grams per kilo, was introduced into the intestines in concentrated solution, 1 gram per kilo of desiccated thyroid in 200 cc. of water was used, 5 grams per kilo of the amino acid, glycocoll, and 0.15 gram per kilo of caffein in concentrated solution.

Cannon and de la Paz (4) have shown that the adrenals are stimulated to an increased output of adrenalin during combat and that the function of this is to produce greater vaso-constriction in the splanchnic area, thus shunting more blood to the muscles where it is needed, especially during combat. If catalase is the enzyme in the body primarily responsible for oxidation, it may be that the increased amount of adrenalin in the blood during combat stimulates the liver to an increased output of catalase thus aiding in increasing oxidation and giving rise to the energy for the fight. Stern (5) and others have shown that the introduction of tetrahydro-β-naphthylamin produces a great rise of temperature with increased heat production and diminished heat elimination, and that this hyperthermia is not reduced by the ordinary antipyretics, but only by deep narcosis. Hultgren and Andersson (6) found that the excision of the adrenals

lowered temperature while Freund and Marchand (7) found that the injection of epinephrin produced fever. Binz (8) found that large doses of caffein produced a slight degree of fever. Means, Aub, DuBois (9), and others have found that caffein increases oxidation. Finkelstein, Chaps and Bingl (10) observed that the hypodermic injections of large amounts of sodium chloride may produce fever. Dahm and Steck (11) found that the ingestion of sodium chloride increased oxidation. This observation has been repeated and confirmed by Tangl (12) on curarized animals with their kidneys removed. Raeder (13) also found that saline injections, especially if hypertonic, increased the respiratory exchange. Freund and Grafe (14) also found that salt fever was accompanied by an increased heat production. Magnus-Levy (15) observed an increased carbon dioxide output in a man fed upon thyroid extract and an increased oxygen intake in cases of exophthalmic goiter. Anderson (16) observed a decrease in metabolism in cases of myxedema as was indicated by a decreased oxygen intake and carbon dioxide output, and that the metabolism was increased to normal by thyroid feeding. We (17) found that the feeding of thyroid to cats greatly increased the catalase of the blood. The increase in catalase described in this paper after the introduction of thyroid into the alimentary tract of dogs suggests that the increase in the catalase of the blood of animals fed thyroid is due to the stimulation of the liver to an increased output of this enzyme. Winternitz (18) found that "the removal of the thyroid gland caused a drop in the catalase activity of the blood which was compensated if thyroid were fed" and that in hyperthyreosis the catalase of the blood tends to increase while in hypothyreosis it assumes a lower level than normal. Becht (19) on the other hand claims that thyroid feeding decreases the catalase of the blood. It should be mentioned in this connection also that Becht holds that narcotics slightly increase the catalase content of the blood, while we found that it produced a great decrease both in vivo and in vitro.[1]

[1] Owing to our proximity it has been suggested and even urged that Dr. Becht and myself carry out some joint experiments in an attempt to clear up the differences in our results. I am sorry to say that Dr. Becht seems to be unwilling to carry out such experiments.

The increase in catalase brought about by the introduction of tetrahydro-β-naphthylamin, caffein, adrenalin, sodium chloride, desiccated thyroid and glycocoll is offered in explanation of the increased oxidation produced by these substances and hence of the accompanying fever in so far as the increased oxidation is involved in this.

It is recognized that narcotics such as chloroform and ether produce a decrease in oxidation and a fall in temperature even of a normal animal. In chart 2 it may be seen that these two

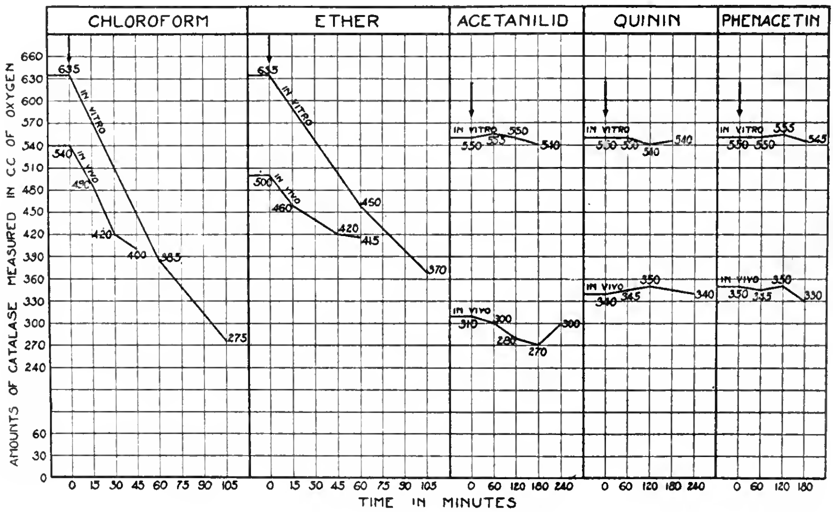

Fig. 2

narcotics produce a decrease in the catalase of the blood in vivo and in vitro. The animals used were cats. The chloroform as well as the ether was administered by bubbling air through these anaesthetics in a bottle which was connected by a rubber tube to a cone adjusted to the snout of the animal. No attempt was made to administer these anaesthetics in equimolecular concentrations, sufficient of the anaesthetic being administered to keep the animal in deep narcosis. The catalase of the blood obtained from the external jugular was determined before as well as at intervals during the administration of the narcotic.

The destruction in vitro was produced by exposing a small amount of defibrinated blood while it was being agitated to chloroform as well as to ether vapor under a pressure of about 15 mm. of mercury. Care was taken that none of the liquid anaesthetic came in contact with the blood since this produces very rapid destruction of the catalase.

Under acetanilid, quinin and phenacetin in chart 2 it may be seen that these substances have no effect on the catalase of the blood either in vivo or in vitro. After opening the abdominal wall of rabbits while under ether, 5 grams per kilo of each of the substances in 75 cc. of water were introduced into the upper part of the intestine. As soon as the substances were introduced, the whole process requiring about three minutes, the wound was sewed up and etherization discontinued. The catalase of the blood of the jugular vein of the rabbits was determined before as well as at certain intervals after the introduction of the substances. The effect in vitro was determined by adding 0.2 gram of acetanilid, quinin and phenacetin respectively to 15 cc. of defibrinated blood. The catalase content of 0.5 cc. of the blood was determined before as well as at certain fixed intervals after the addition of the substances.

In chart 3 is shown the effect produced by chloroform, ether, acetanilid, quinin and phenacetin on the catalase of the blood of the liver as well as of the jugular. The animals used were dogs and cats and the chloroform and ether were administered by means of a cone adjusted to the snout of the animal as already described. Two grams per kilo in 300 cc. of water of acetanilid, quinin and phenacetin were introduced into the upper part of the intestines of dogs. The continuous line curves were constructed from data obtained from the blood of the liver, the discontinuous line curves from the blood of the portal, and the dotted line curves from the blood of the jugular.

It may be seen that the catalase of the blood of the liver which is normally higher than that from any other part of the body thus indicating that catalase is continually being given off from the liver into the blood was decreased by chloroform, so that at the end of fifteen minutes it was the same as that of the

jugular blood. This is taken to mean that chloroform had reduced the liver function so far as catalase production was concerned to nil. Under ether it may be seen that although the catalase of the blood was decreased, due to the direct destruction of the enzyme by the ether, the liver so far as catalase production is concerned was still functioning as is indicated by the fact that the liver blood continued during the anaesthesia to be richer in catalase than the blood of the jugular. The decrease in catalase is offered in explanation of the decreased oxidation

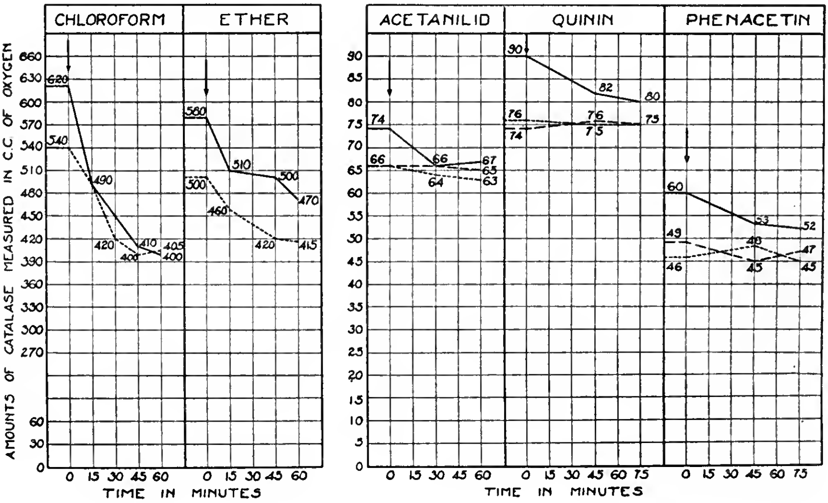

FIG. 3

which undoubtedly plays a part in bringing about the fall of temperature during anesthesia.

Under acetanilid, quinin and phenacetin it may be seen that while these substances did not decrease the catalase in the blood of the jugular and portal veins there was a tendency to decrease the catalase of the blood of the liver, thus indicating that these substances were disturbing the liver function so far as catalase production was concerned very much as chloroform did, but not so extensively.

In figure 1 it may be seen that tetrahydro-β-naphthylamin

stimulated the liver very strongly to an increased output of catalase which was offered in explanation of the increased oxidation and in part at least for the fever produced by this substance which can not be reduced by antipyretics such as acetanilid, quinin and phenacetin, but only by strong narcotics. Why can not an antipyretic such as acetanilid reduce the fever produced by an substance such as tetrahydro-β-naphthylamin while a strong narcotic such as chloroform can? The answer that suggests itself is that chloroform destroys catalase more rapidly than tetrahydro-β-naphthylamin stimulates the liver to a production of it, resulting in a decrease in catalase and hence in oxidation which is probably responsible for the lowered temperature. Acetanilid on the other hand being very weak as a catalase destoyer, does not decrease this enzyme sufficiently to counterbalance the increase produced by tetrahydro-β-naphthylamin, hence in the antagonistic action of these two drugs there results an increase in catalase with resulting increase in oxidation which is offered in explanation for the rise in temperature produced by this drug.

SUMMARY

1. Tetrahydro-β-naphthylamin, adrenalin, caffein and sodium chloride stimulate the alimentary glands particularly the liver to an increased output of catalase which is offered in explanation of the increased oxidation produced by these substances and hence for the accompanying fever in so far as the increased heat production is involved in this.

2. Chloroform decreases catalase both by decreasing its output from the liver and by direct destruction of this enzyme. Ether decreases catalase principally by direct destruction of the enzyme without disturbing the liver function so much as does chloroform. Acetanilid, quinin and phenacetin produce a slight decrease in catalase by decreasing its output from the liver.

3. Chloroform and ether lower temperature in so far as decreased oxidation is involved in this by decreasing catalase, the enzyme principally responsible for oxidation in the body. The

fact that acetanilid, quinin and phenacetin have little or no effect in decreasing catalase suggests that their mode of action in lowering temperature is not due to a decrease in oxidation.

REFERENCES

(1) Stuhlinger: Archiv. für experimentelle Pathologie und Pharmakologie, 1899, xliii, 167.
(2) Gottlieb: Archiv. für experimentelle Pathologie und Pharmakologie, 1891, xxviii, 167.
(3) Burge: The American Journal of Physiology, 1919, 48, no. 2, 133.
(4) Cannon and de la Paz: The American Journal of Physiology, 1911, xxviii, 64.
(5) Stern: Arch. f. path. anat. u. Physiol., 1889, cxv, 14. Ibid., 1890, cxxi, 376.
(6) Hultgren and Andersson: Sollman's Pharmacology, 1917, 333.
(7) Freund and Marchand: Arch. exp. Path. Pharm., 1913, 72, 56.
(8) Binz: Archiv. f. Pathologie und Pharmakologie, 1878, 9, 31.
(9) Means, Aub, Du Bois: Archives Int. Med., 1917, xix, 832.
(10) Finkelstein, Chaps and Bingl: Arch. exp. Path. Pharm., 1910, 64, 1.
(11) Dahm and Steck: Physiologische Gesellschaft zu Berlin verhandlungen, 1909, xxxiv.
(12) Tangl: Biochem. Zeitschr., 1911, xxxiv, 1.
(13) Raeder: Biochem. Zeitschr., 1915, lxix, 257.
(14) Freund and Grafe: Arch. exp. Path. Pharm., 1911, lxvii, 55.
(15) Magnus-Levy: Berl. klin Wochenschr., 1895, xxx, 650.
(16) Anderson: Hygeia, Stockholm, 1898 (quoted in Tigerstedt's Lehrbuch der Physiologie).
(17) Burge, Kennedy, Neill: American Journal of Physiology, 1917, 43, no. 3, 433.
(18) Winternitz: Archives Int. Med., 1911, vii, 625.
(19) Becht: American Journal Physiology, 1919, xlviii, 171.

DRUGS AFTER CHLORINE GASSING

III. NOTES ON THE TREATMENT OF GASSED DOGS WITH CALCIUM, WITH QUININE, AND WITH ATROPINE

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Received for publication July 22, 1919

Dogs previously exposed to minimal lethal concentrations of chlorine gas (800–900 parts per million, by volume) have been treated with subcutaneous injections of certain drugs; calcium lactate, quinine hydrochlorid and atropine sulfate. The essential features of these experiments are presented below in tabulated form.

CALCIUM

Calcium treatment was tested on account of its alleged favorable influence upon certain types of lung edema (1).

QUININE

Quinine was tested in the hope that it might exert a favorable influence upon the autolysis in the injured lung tissue or by other depressant actions.

ATROPINE

The favorable influence of atropine reported by Cow (2) upon gassed rabbits led us to make a few trials in dogs.

[1] Published with the permission of the Director of the Chemical Warfare Service.

TABLE 1

Calcium injections in gassed dogs

NUMBER	SPECIES	WEIGHT	CHLORINE	Ca LACTATE, EACH DOSE	NUMBER OF DOSES	INTERVAL BETWEEN DOSES	SURVIVED	REMARKS
		kilos	*p. p. m.*	*mgms. per kilo*		*hours*	*hours*	
A1	Terrier	9	833	20	3	6	12–13	
A3	Mongrel	15	815	20	3	6	12–20	
A6	Bull	14	725	20	1		1–3	
A7	Collie	11	729	20	2	9	10–18	
A8	Collie	18	759	20	3	$3\frac{1}{2}$	7–10	
A9	Collie	27	865	20	2	2	$2\frac{1}{2}$–4	
A22	Mongrel	11	840	50	5	4	24	
A24	Terrier	9	869	50	2	3	5	
A25	Hound	$11\frac{1}{2}$	829	50	7	3	28	
A27	Spaniel	9	846	50	2	2	5	
A28	Hound	9	848	50	13	3	51	
A21	Terrier	9	843	100	10	6	70	
A29	Hound	11	888	100	2	6	11–19	
A30	Collie	13	846	100	2	8	9–17	
A70	Hound	24	826	100	1		4	
A71	Mongrel	$12\frac{1}{2}$	950	100	1		12–20	
A31	Terrier	$13\frac{1}{2}$	864	200	1		7–20	Intratracheal injection
A32	Mongrel	16	861	200	1		26	Intratracheal injection
A33	Hound	11	832	200	1		3	Intratracheal injection
A34	Mongrel	11	859	200	1		3	Intratracheal injection
A16	Mongrel	10	821	200	3	6	13–21	
A35	Bull	15	857	400	2	12	12–20	
A36	Bull	$16\frac{1}{2}$	856	400	1		6–20	
Average..							16.4	

TABLE 2

Quinine injections in gassed dogs

NUMBER	SPECIES	WEIGHT	CHLORINE	QUININE-HCl, EACH DOSE	NUMBER OF DOSES	INTERVAL BETWEEN DOSES	SURVIVED
		kilos	*p. p. m.*	*mgms. per kilo*		*hours*	*hours*
A10	Mongrel	11	807	50	4	4	23
A11	Mongrel	18	862	50	2	3	3–5
A12	Mongrel	13	846	50	5	$2\frac{1}{2}$	12–22
A19	Mongrel	10	883	100	2	6	7–12
A38	Bull	$16\frac{1}{2}$	880	100	1		5
A39	Hound	$15\frac{1}{2}$	869	100	1		5
A14	Bull	11	875	200	1		4
A15	Mongrel	10	864	200	1		3
Average..							8.8

TABLE 3

Atropine injections in gassed dogs

NUMBER	SPECIES	WEIGHT	CHLORINE	ATROPINE SULFATE	SURVIVED	REMARKS
		kilos	*p. p. m.*	*mgms. per kilo*	*hours*	
A49	Terrier	11	845	0.2	14	
A48	Mongrel	13	851	0.5	4	
A50	Mongrel	11	848	0.5	4	Injection before gassing
A46	Mongrel	11	880	1.0	3	Injection before gassing
Average..................................					6.2	

SUMMARY

No evidence was obtained favorable to the employment of subcutaneous injections of calcium lactate in edema of the lung. Calcium was tested in twenty-three, quinine in eight, and atropine in four gassed dogs, without a single recovery. It is improbable that any of these substances can be used to advantage in this condition.

REFERENCES

(1) Chiari and Januschke: Arch. exp. Path. u. Pharm., 1910, lxv, 120.
(2) Cow, D.: Lancet, 1915, clxxxviii, 1147.

LOCAL ANESTHETICS: DO THEY PRECIPITATE PROTEINS?

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Precipitation of proteins would be an important property for local anesthetics, in that it might lead either to a desirable astringent action, or to undesirable irritation. The following experiments show, however, that most of the anesthetics now available do not precipitate proteins, and none did so to a serious degree.

Method. The aqueous solution of the anesthetic (2.5 cc.) was poured on 5 cc. of the protein solution. This was done slowly, with a view to possible ring tests, but none occurred. The solutions were then shaken.

The following filtered protein solutions in 0.9 per cent NaCl were used: "Albumen" = a 10 per cent solution of moist egg white. "Serum" = 1 per cent of dried serum. "Peptone" = 1 per cent of Witte's peptone.

The experiments were made by Mr. O. H. Schettler. The results may be grouped as follows:

A. Saturated solutions of "insoluble" anesthetics. These were made from 1 per cent suspensions.

Anesthesin Cycloform Orthoform-new Propaesin	No precipitate with any of the proteins.

B. 1 per cent solutions of soluble anesthetics.

Alypin (hydrochlorid) Cocain (hydrochlorid) Holocain (hydrochlorid) Procain (hydrochloride)	No precipitate with any of the proteins.

Apothesin (hydrochlorid): Precipitated with albumen, not with serum or peptone.

C. 5 per cent solutions of soluble anesthetics.

Alypin (hydrochlorid) Cocain (hydrochlorid) Procain (hydrochlorid)	No precipitate with any of the proteins.

Apothesin hydrochlorid: Turbidity with albumen and opalescence with serum; no change with peptone. The precipitates do not clear on acidulation with HCl.

D. 5 per cent acidulated solutions of the "insoluble" anesthetics. The anesthetics were brought into solution by a minimum of diluted HCl.

Cycloform Orthoform—new Propaesin	These do not precipitate any of the proteins. Precipitation does occur when the solutions are mixed, but it redissolves on the addition of acid, and is therefore due to the precipitation of the anesthetics by the alkalinity of the proteins.

Anesthesin: Turbidity (not cleared by acid) with serum. No precipitation of albumen or peptone.

It may be remarked that the neutral and acid *orthoform* solutions turned yellow on standing; the color deepening to reddish brown on acidulation with hydrochloric acid.

THE RÔLE OF THE BROMIDE SALTS ON RHYTHMICALLY CONTRACTING ORGANS

I. THE ACTION OF THE BROMIDES ON THE ISOLATED MAMMALIAN HEART[1]

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Although the general bromide reactions on the heart are now fairly well understood, less seems to be known of their detailed behavior, such as may be observed on the isolated mammalian heart. In a previous report the author with Prof. C. W. Greene (1) showed that a perfused frog heart is slightly augmented in rate and later slightly decreased by a bromide substituted Ringer's solution. Such a Ringer's solution is just as effective as the ordinary chloride Ringer's solution for maintaining the rhythmicity of the frog heart. The following experiments were performed to determine whether the isolated mammalian heart behaved in a similar manner.

The older literature, as reviewed by Krosz (2), shows that the heart is depressed by the bromides. These reports are not entirely trustworthy in interpretation since they are essentially based upon results obtained by applying concentrated potassium bromide solutions on the surface of the heart. By such a method the typical bromide reaction is obscured by the depressant potassium action and by osmotic effects. Other reports are based

[1] The facts presented in this paper were used as a part of a thesis rendered for the degree of Doctor of Philosophy, at the University of Missouri, under the direction and advice of Prof. C. W. Greene to whom I express my gratitude.

This investigation was partly supported in 1914 by a grant from the Committee on Research of the Council on Pharmacy and Chemistry of the American Medical Association.

on clinical cases which obviously are obscured by the bromide reaction on the central nervous system. Although Krosz attributed the depression of the frog heart to the potassium factor he believed nevertheless that much was due to the bromide factor.

In recent years, it has been established by Loeb on marine animals (3) and by Lingle (4), Benedict (5) and others that the bromides are stimulating upon the rhythmic muscular tissues, such as the turtle heart if the sodium salt is used in isotonic concentrations. In a previous report (6) I have indicated that smooth muscle is similarly stimulated. In the skeletal muscle of the frog, Loeb (7) has observed stimulation by the bromides. But in the perfused rabbit heart Busquet and Pachon (8) have found sodium bromide non-toxic.

METHOD

The heart of a cat or a dog was excised in the usual manner and was suspended in a perfusing apparatus designed in this laboratory. The coronaries were perfused with a Ringer's solution and was compared with a similar solution in which the chloride salts were replaced by the corresponding bromide salts in equimolecular concentrations. Both the chloride and the bromide solutions were diluted with corresponding quantities of defibrinated blood taken from the same animal.

The usual plan of experiment was to establish rhythmicity by the chloride solution and then to test the bromide reaction by perfusion for short intervals, usually three minutes, and then allowing the heart to recover by the chloride perfusion.

EXPERIMENTAL RESULTS

1. The effect of the bromide perfusion on the isolated mammalian heart

Perfusion of the isolated mammalian heart by the bromide substituted Locke's or Ringer's solution produces at first an increase in the rate and the amplitude of the contractions and

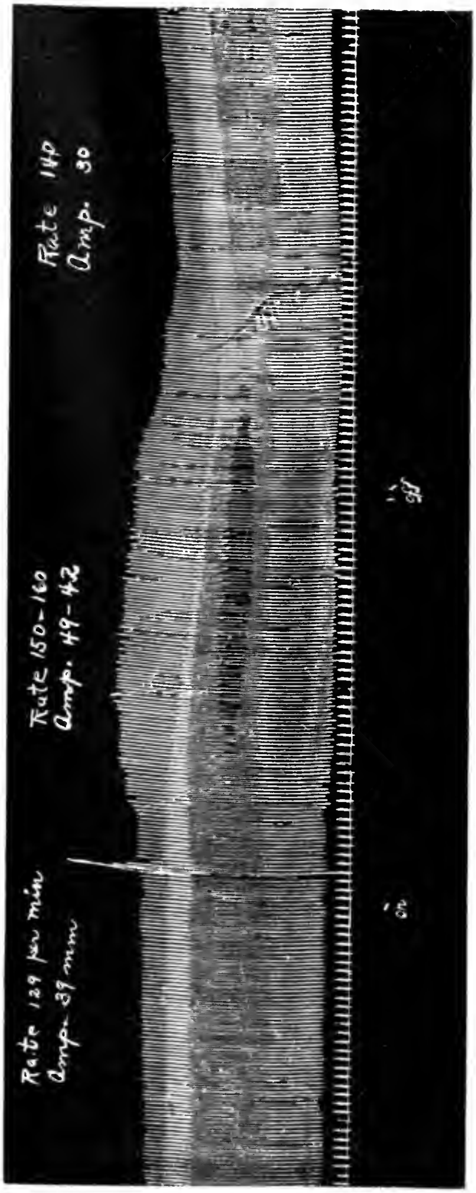

FIG. 1. BROMIDE LOCKE PERFUSION OF THE ISOLATED HEART OF A PUPPY

This shows a mild effect of a bromide Locke's perfusion. Time in seconds.

is followed within one to three minutes by a decrease. The increase in the amplitude falls on both the systole and the diastole of the contraction, figures 1 and 3. During the period of de-

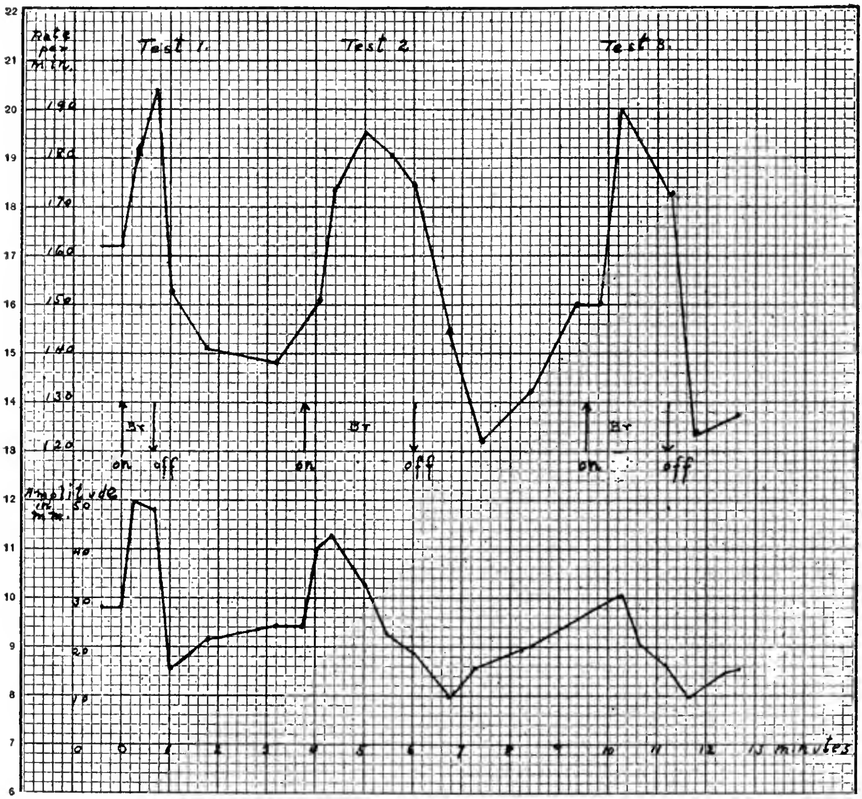

Fig. 2. A Graph Representing Alternate Perfusion of a Chloride and a Bromide Locke's Solution on the Isolated Heart of a Puppy

This shows the marked increase in the rate and the amplitude during a bromide perfusion and the secondary decrease following it. Note that the amplitude decreases while the rate is still increasing. The space between the arrows "on" and "off" indicates the bromide perfusion.

pression the perfusion rate is greatly decreased due to the constriction of the coronaries.

In some experiments after a certain depressive effect was reached there occurred a marked reaction at the instant when

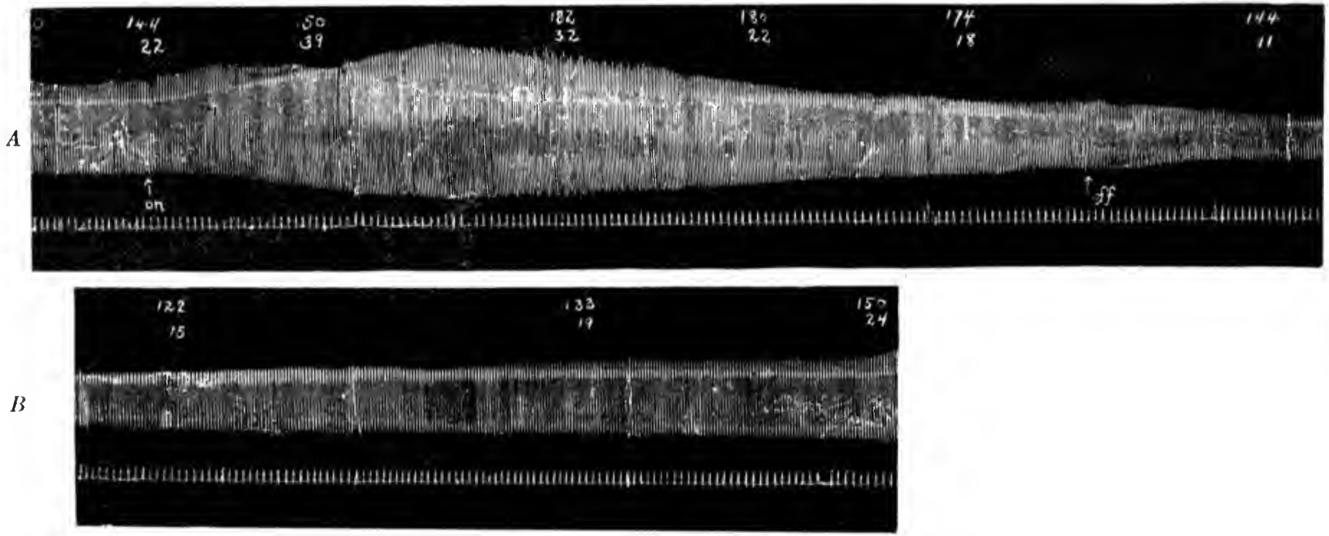

Fig. 3. Bromide Locke Perfusion of the Isolated Heart of a Puppy

B shows the recovery of *A*. Note the secondary decrease after the primary increase in both rate and amplitude. Also note the sudden decrease after the bromide solution is replaced by the chloride solution. Time in seconds.

the perfusion was changed from the bromide solution to the chloride solution. This was manifested by a sudden decrease in the rate and the amplitude of the contraction greater than would have occurred had the perfusion not been changed, figure 3. Sometimes this reaction did not take place particularly when the bromide solution was removed before depression had set in. The typical response is illustrated in figure 2 in which an

FIG. 4. A GRAPH REPRESENTING A CONSTANT PERFUSION OF A BROMIDE RINGER'S SOLUTION ON A CAT'S HEART

This shows the eventual decrease after the primary augmentation and the beneficial influence of increasing the perfusion pressure (arrows).

experiment is plotted and in figure 3 in which the figures written in at the top of the trace designate the rate per minute and amplitude in millimetres, respectively. These show that the rate and the amplitude of the heart increased at once during the bromide perfusion and passed quickly into a state of depression in which the amplitude decreased while the rate was still increasing.

The secondary depression may be explained in part by the constriction of the coronaries, since an increase in the perfusion pressure retards the depression for a short time. Soon after the appearance of the secondary depression, arrhythmia occurs which can be removed for a time by a similar increase in the perfusion pressure, but the coronaries tend to constrict still more and the same depression is repeated, figure 4.

In three experiments the heart was revived from the start by a bromide Ringer's solution. One of these experiments is represented by a graph in figure 4. It shows the decreased heart activity being interrupted after each increase of perfusion pressure. Coincident with the decrease in activity there occurs a decrease of the perfusion rate. These hearts remained rhythmic from ten to eighteen minutes. Control experiments with a chloride Ringer's solution maintain hearts in rhythm for several hours.

2. *Uniformity of results*

To show the relative uniformity of the results all of the tests are summarized according to the effect, shown in table 1. Those experiments not showing depressive effects were due to the shortness of the test. In the cat heart the early stimulation was very short in duration and was absent in many tests. When a heart was tested repeatedly with a bromide Ringer's solution it was found that the later tests were less intense, figure 5. This

TABLE 1

Table showing the frequency of change in the rate and the amplitude of the contractions in dog and cat hearts calculated for all experiments

		PRIMARY INCREASE	NO CHANGE	PRIMARY DECREASE	EVENTUAL DECREASE
		per cent	*per cent*	*per cent*	*per cent*
Dog*	Rate	85.7	5.7	8.6	65.0
	Amplitude	68.6	8.6	22.8	90.0
Cat†	Rate	39.0	9.0	52.0	80.0
	Amplitude	36.0	14.0	50.0	79.0

* Including 35 tests.

† Including 25 tests.

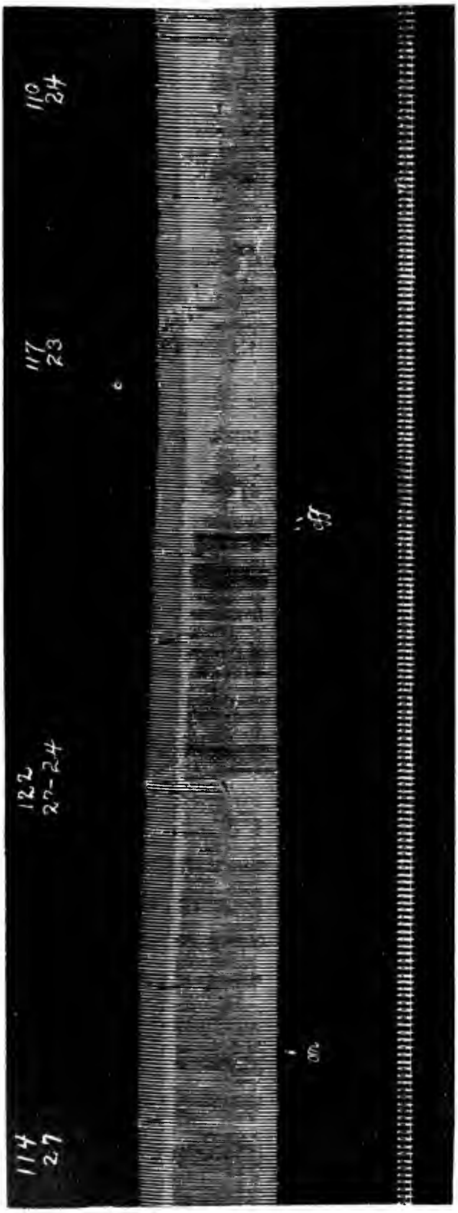

Fig. 5. Bromide Locke Perfusion of the Isolated Heart of a Puppy

Showing that later perfusion tests act less intense. Note the rate is increased but the amplitude is reduced. (Same heart and solution as in figure 3.)

change in the behavior may be the manifestation of a surface action caused by the gradual substitution of the chlorides in the heart muscle in contrast to the sudden substitution occurring in the earlier tests.

DISCUSSION

The constriction of the coronaries seems to be the principal cause for the depressive effect of a bromide perfusion through the mammalian heart. This constriction need not be looked upon as the initiation of a new process but is an acceleration of a process taking place in all perfused hearts. Cushny and Gunn (9) described such an effect on the coronaries by blood serum or plasma. They attributed this effect as due to the alteration of the heart in the isolated condition. Morawitz and Zahn (10) and Hermel (11) found that old perfused blood and standing defibrinated blood have this constrictor influence on the coronaries when the heart is perfused. Hermel believed that a new colloidal arrangement of the plasma was a possible explanation.

In later experiments (12) it was found that smooth muscle showed an increase in tonicity when suspended in a bromide Ringer's solution. By direct observation on the coronary outflow and by analogy on other smooth muscle tissues it was concluded that the stimulation of the smooth muscle of the coronaries leads to a depression of the heart through asphyxiation. This view does not exclude the possibility of a later depression by action directly on the heart muscle by the bromide ions.

The early stimulation of the heart muscle seems to be the characteristic reaction typical for most motor types of protoplasm, since all three forms of muscular tissues are similarly affected. In the coldblooded animals the depression is less intense than in the mammalian species possibly because the former type of animals thrive on a greater economy of oxygen. Recently Macht and Hooker (13) have shown that perfusion of the medulla of a mammal by a bromide solution stimulates the respiratory and cardio-inhibitory center. It is therefore evident that the bromides are more or less irritating to most tissues of the body.

Only later do symptons of depression occur caused perhaps by a disturbance of the oxidative mechanism. It is noteworthy in this connection that the central nervous system of the frog is not depressed by sodium bromide.

The augmentation of the heart by the bromides in the intact animal is not pronounced for several reasons. First, the concentration of the bromides used in our experiments is greater than can be realized in the blood of the intact animal, since isotonic concentrations were used. Secondly, the early action of the bromides on the cardio-inhibitory center counteracts the stimulation of the heart. Thirdly, isolation of the heart produces changes in the heart probably due to the early asphyxiation so that this influence is more prominent in the isolated condition. Such an alteration has been indicated by Guthrie and Pike (14) who showed that the isolated heart responds readily to pressure changes whereas the heart *in situ* fails to do so even after all extrinsic nerves are cut.

SUMMARY

1. The action of the bromides on the perfused isolated mammalian heart is characterized by a primary increase followed by a decrease in the rate and the amplitude of the contractions.

2. The depression occurs more quickly on the amplitude than upon the rate.

3. The secondary depression is looked upon as being due to the constriction of the coronaries. This view does not exclude the possibility of a direct influence of the bromide ion.

4. The withdrawal of the bromide perfusion from the heart leads to a sudden depression greater than would have occurred if the bromide perfusion had been continued.

5. A bromide Ringer's or Locke's solution is inadequate to maintain rhythmicity in a perfused isolated mammalian heart for as long a time as a similar chloride solution. In the frog heart however the bromide Ringer's solution is at least as efficient as a chloride Ringer's solution.

REFERENCES

(1) Greene and Kruse: Journ. A. M. A., 1913, lxi, 271.
(2) Krosz: Arch. exp. Path. Pharm., 1876, vi, 1. (Older literature.)
(3) Loeb: Amer. Journ. Physiol., 1900, iii, 327.
(4) Lingle: Amer. Journ. Physiol., 1901, iv, 272.
(5) Benedict: Amer. Journ. Physiol., 1905, xiii, 192.
(6) Kruse: Amer. Journ. Physiol., 1917, xlii, 595.
(7) Loeb: Fick's Festschrift, Braunschweig, 1899.
(8) Busquet and Pachon: Journ. de Physiol., 1909, xi, 243.
(9) Cushny and Gunn: Journ. Pharmacol. and Exp. Ther., 1913, v, 1.
(10) Morawitz and Zahn: Arch. klin. Med., 1914, cxvi, 364.
(11) Hermel: Arch. klin. Med., 1914, cxv, 207.
(12) Kruse: Loc. cit.
(13) Macht and Hooker: Journ. Pharmacol. and Exp. Ther., 1918, xi, 63.
(14) Guthrie and Pike: Amer. Journ. Physiol., 1907, xviii, 14.

II. THE ACTION OF THE BROMIDES ON SMOOTH MUSCLE[1]

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In a previous communication (1), the author showed that the coronaries of the heart are constricted by a perfusion of a bromide Ringer's solution. The following experiments were performed to determine whether other types of smooth muscle show augmented activity under bromide treatment.

Stiles (2) showed that isolated oesophagus strips of the frog are maintained in rhythm by a bromide Ringer's solution. Prochnow (3) showed that the sodium halides increase the tonus of the uterus and the smooth muscle of arteries in the order of fluorides, iodides and bromides. The rate and power of the contraction were not found affected, but the excitability was found increased. Very dilute solutions were used. Winkler (4) showed that sodium bromide like sodium chloride at first increases the irritability of the frog stomach and later decreases it. He used N/20 and N/10 sodium bromide and sodium chloride solutions in the pure form.

METHOD

Smooth muscle strips or segments taken from the intestine of a cat or dog were suspended in an oxygenated chloride Ringer's solution at 38°C., according to the method of Magnus (5).

[1] A preliminary report of this investigation was read before the American Physiological Society, December, 1916. Amer. Journ. Physiol. xlii, no. 4, 1917.

The facts presented in this paper were used as a part of a thesis rendered for the degree of Doctor of Philosophy, at the University of Missouri, under the direction and advice of Prof. C. W. Greene to whom I express my gratitude.

After rhythmicity was established the tissue was tested with a bromide Ringer's solution containing equimolecular quantities of bromides for the chlorides. Sometimes two segments were taken from adjacent portions of the intestine and were mounted, one in a chloride and the other in a bromide Ringer's solution. After variable intervals these solutions were interchanged, thus controlling the activity of a strip with another as well as controlling identical solutions with more than one strip.

The test solutions were always tested by the freezing point method for its molecular concentration and by Mohr's method for its halide concentration. The tissues were taken principally from the intestines of cats and dogs. Some tests were made on the fallopian tubes of the dog, the oesophagus, stomach and intestines of the frog, and oesophagus, stomach and oviduct of the turtle. If the organ was small it was mounted as a segment in a longitudinal manner. Larger organs were cut into narrow strips or circular segments and mounted as such.

RESULTS

Intestinal segments suspended in a bromide Ringer's solution show an augmentation in rate, amplitude and tonicity. Changes in rate and amplitude were variable but changes in tone were nearly always observed.

Rhythmicity. When two strips or segments of intestine were mounted at the same time, one in a chloride and the other in a bromide Ringer's solution, the bromide strip was the first to initiate rhythmicity. If the solutions were then interchanged the inactive strip became active and the active strip became inactive or showed decreased activity, figure 1. This reaction was repeated a number of times.

Sometimes an arrhythmic segment became perfectly rhythmic in the bromide Ringer's solution, figure 2. Since solutions containing a small quantity of blood showed better rhythmicity in both halide solutions, the regularizing influence of the bromide solution was associated with more perfect tissue respiration brought about either by an increased activity of the oxidative

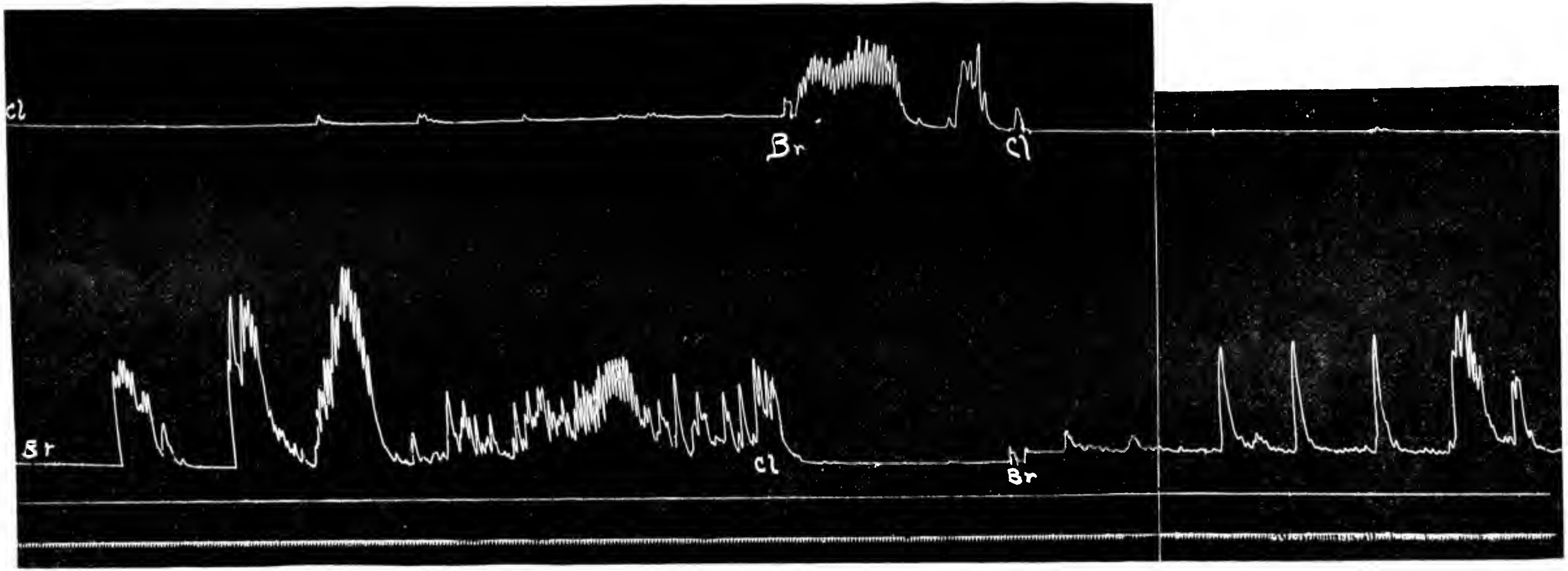

Fig. 1. Two Segments of Cat Intestine

There is an interval of thirty seconds between the two figures. The top segment was mounted in a chloride Ringer's solution and the lower segment in a bromide Ringer's solution. The solutions were interchanged at the marks in the third line. The bromide solution caused an augmentation in each case. Temperature 38°C. Time in five seconds.

enzymes or by a facilitation in the oxidative mechanism. Later experiments showed that catalase activity is increased by the bromides.

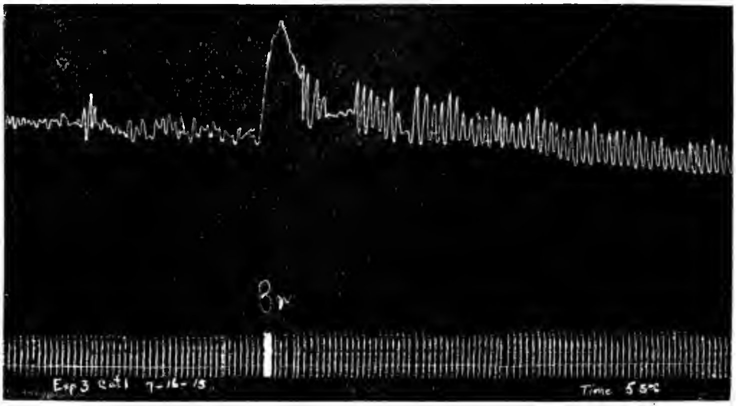

FIG. 2. INTESTINE OF CAT, SHOWING THE REGULARIZING INFLUENCE OF THE BROMIDE SOLUTION

The segment was immersed in a bromide Ringer's solution at the mark. Note the primary increase of tone. Temperature 38°C. Time in five seconds.

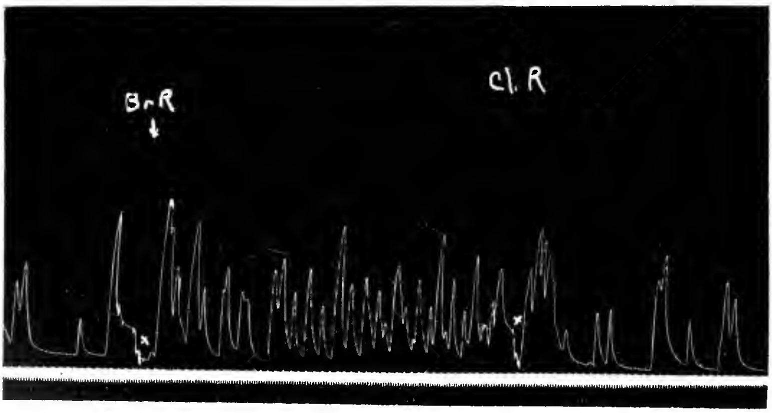

FIG. 3. INTESTINE OF CAT, SHOWING THE STIMULATING INFLUENCE OF A BROMIDE RINGER'S SOLUTION

Note the reversibility of the reaction. Temperature 37°C. Time in two and one-half seconds.

The increase in the rate was as high as 20 per cent in many experiments but was very slight when the pre-existing rhythm was regular. The marked increases of rate were always associated with an irregular pre-existing rhythm, figure 3.

Amplitude. Changes in the amplitude were inconstant and slight. A sluggishly acting intestine showed the more marked increases in amplitude. Very active intestinal segments showed practically no further changes in amplitude. If the tonicity

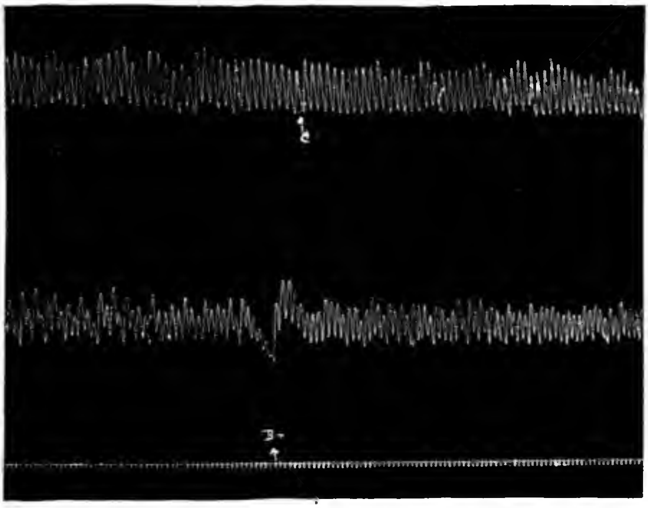

FIG. 4. TWO SEGMENTS OF CAT INTESTINE, SHOWING THAT THE AUGMENTATION OF A BROMIDE SOLUTION IS LESS INTENSE IF THE SEGMENT IS RHYTHMIC

The top segment is in bromide and the lower segment in chloride Ringer's solution. At the mark the solutions are interchanged. Note the augmentation of rate produced in the bromide solution. Time in five seconds.

change was very great the amplitude was sometimes decreased. The most marked increases in amplitude were associated with moderate tone changes.

Tone. An arrythmic segment responded by a marked increase of tone in the bromide solution. A high degree of tone was rarely long maintained. It was usually followed by a decrease simulating fatigue. Sometimes it really decreased below the previous chloride level. If the tissue was immersed at this

stage in chloride solution, the tone decreased to a still lower level showing that the tissue was still augmented in the bromide solution. Sometimes the secondary decrease was absent. Such cases however were the exception.

A very rhythmic segment showed occasionally only slight change in tone and indeed, sometimes was just perceptible, figure 4. The response was in all cases dependent on the previous activity of the tissue.

THE EFFECT OF THE BROMIDES ON OTHER SMOOTH MUSCLE TISSUES

Fallopian tube of the dog. The fallopian tube of the dog subjected to a bromide Ringer's solution responded essentially as the intestine of the cat and dog with the exception that the tone was better maintained during the primary rise.

Smooth muscle of the frog. The results on the oesophagus, stomach and intestine of the frog were inconstant. It was indeed difficult to detect changes when these segments were changed from the chloride to the bromide Ringer's solution. In exceptional cases augmentation was observed.

Smooth muscle of the turtle. The oesophagus, stomach and intestine of the turtle seemed little affected by the bromide solution just as it was in the frog. The oviduct of the turtle simulated the results found characteristic in the mammalian intestine but were less intense.

Point of action. Plexus free preparations were made according to the method of Magnus (6), with and without the application of atropine to determine whether the bromide augmentation was due to a stimulation of either muscle or nerve elements or due to a stimulation of both. Augmentation was observed in plexus free preparations with atropine but was of less magnitude than when the plexus was intact. The toxic effect of atropine on the muscle is probably in part responsible for the decrease. The conclusion was reached that both muscle and nerve are stimulated by the bromides in plexus containing organs. Prochnow (7) concluded that the bromides stimulate smooth muscle directly, on the basis of experiments on the carotid artery of ox. Such preparations are reported to be free from intrinsic nerve cells.

DISCUSSION

In a previous communication it was shown that the bromides act as mild stimulants to skeletal muscle, cardiac and smooth muscle of the heart and to certain medullary nerve centers (8). In this paper it is shown that many types of smooth muscle are stimulated. The early effect of the bromides therefore appears to be a primary stimulation.

Symptoms of stimulation have been observed clinically (9) both on the central nervous system and on the gastro-intestinal tract. Depression is a later manifestation.

The variable quantitative changes occurring in the tissue are dependent on its physiological condition. In the strip technique the tissue goes through variable degrees of asphyxiation. A mild stimulant has accordingly a profound effect. The same principle is evident in fatigued muscle nerve preparations of a frog. It is for this reason that segments contracting arrhythmically show the most profound changes whereas the changes are less intense in rhythmic preparations.

SUMMARY

1. The bromides stimulate smooth muscle slightly when the tissue is in a normal and rhythmic condition and markedly when it is arrhythmic or in a partly fatigued state.

2. The primary stimulation of the bromides seems to be a property common for many tissues.

REFERENCES

(1) Kruse: Journ. Pharmacol. and Exp. Ther., 1919, xiv, 63.
(2) Stiles: Amer. Journ. Physiol., 1901, iv, 272.
(3) Prochnow: Arch. internat. de Pharmacod. et de Therap., 1911, xxi, 287.
(4) Winkler: Pflueger's Arch., 1898, lxxi, 357.
(5) Magnus: Pflueger's Arch, 1904, cii, 123.
(6) Magnus: Pflueger's Arch., 1904, cii, 349.
(7) Prochnow: Arch. internat. de Pharmacod. et de Therap., 1911, xxi, 287.
(8) Kruse: Journ. Pharmacol. and Exp. Ther., 1919, xiv, 63.
(9) Fere: Nouvelle Iconographie de la Salpêtrière, 1902, xv, 435.
Mitchel: Therap. Wochensch., 1896, iii, 821.
Hodges: South. Med. Record, Atlanta, 1896, 505.

THE PLASMA AND BLOOD CLOTTING EFFICIENCY OF THROMBOPLASTIC AGENTS IN VITRO AND THEIR STABILITY[1]

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I. INTRODUCTION

It was previously shown (1) that the entire group of hemostatic agents is rather limited in their usefulness, due in part to the difficulty of applying crucial tests in determining their efficiency under actual conditions of wound hemorrhage. This is particularly true of the thromboplastic type of agents derived from tissues or blood. Among these are kephalin, and such commercial preparations as thromboplastin, Coagulen, Hemostatic Serum and Coagulose. The claims made for the hemostatic qualities of these appear exaggerated when it is borne in mind that hemorrhages of whatever origin are most capricious, and generally tend to cease spontaneously, even in hemophilia.

[1] This research was supported in part by a grant from the Therapeutic Research Committee, Council of Pharmacy and Chemistry of the American Medical Association.

There is no doubt that kephalin, at least, accelerates the clotting of oxalate and peptone plasma in the presence of fresh serum in vitro, and it is largely on this basis that its use in hemorrhages has been advocated, although, of course, this does not necessarily follow. Lack of success with these agents has been attributed to the use of old and deteriorated products and it is conceivable also that their activity varies with the dosage and concentration.

It is the object of this communication to compare the thromboplastic activity of several different products; the activity of fresh and old preparations, and the relation of concentration to their activity in vitro. Subsequent communications will deal with hemostatic properties in different kinds of hemorrhages in animals, and toxicity.

The thromboplastic agents tested were secured from various sources on the market, from the manufacturers direct, and two preparations of kephalin were made in the laboratory. These may now be described.

1. Description of the thromboplastic agents tested

Most of the commercial thromboplastic preparations are not secret or patented remedies, although the chemical composition of some, particularly as to active constituents, is vague or unknown. These have been evolved or inspired by reputable investigators in connection with studies on the coagulation of blood. For convenience, the various agents may be conveniently classified into three groups.

1. Thromboplastins. These are saline extracts usually of brain, containing a large quantity of protein, presumably also some kephalin, possibly ferments, salts, etc. Thromboplastin was first introduced by Hess (2). Two such preparations are now on the market under the name of Thromboplastin.

2. Kephalin. This is a lipin (monoamino phosphatid) apparently of fairly definite chemical composition. Bang gives the formula as $C_{42}H_{80}NPO_4$, but according to Hugh MacLean considerable discrepancy exists with respect to the percentage con-

tent of carbon and hydrogen as indicated by the work of various investigators. It is prepared by ether extraction of brain (pig or sheep). It was originally described by Thudicum (3) and is regarded by Howell as the constituent of tissue juice responsible for the coagulation of blood. A mixture of oxalate plasma and fresh serum will clot without kephalin, but the time of clotting is greatly shortened by kephalin. It is, therefore, an accelerator of clotting. Kephalin is not appreciably present in blood, but is abundantly found in tissues, particularly the brain. According to Howell the principal rôle of kephalin is to inactivate or neutralize antithrombin allowing the prothrombin in the presence of calcium to form thrombin which in turn converts fibrinogen to fibrin, the clot. The mechanism of action may be represented schematically as follows from the actual ingredients used in Howell's test for thromboplastic activity:

Oxalate plasma *Serum* *Tissue juice* (*or saline*).

Prothrombin = Thrombin Calcium

Fibrin = Fibrinogen (Thrombin?) Kephalin
(Clot)
Antithrombin

Other theories of blood coagulation, notably those of Morawitz and Hammarsten (5), are based on different conceptions. However, it is not the object of this paper to discuss the theories of blood coagulation. For these the current textbooks of physiology and works of the respective investigators may be consulted. The chemical and physical properties of kephalin are described in monographs by Bang (3) and Hugh MacLean (6). It should be borne in mind that kephalin is insoluble in water, but swells like lecithin, forming an emulsion or colloidal suspension, which is modified by various factors. Owing to this the kephalin content of aqueous (normal saline) extracts of tissues must be variable and uncertain.

3. Serum products. To this class belong preparations known as Coagulen, Hemostatic Serum and Coagulose. These are prepared from sera of different animals, usually, from horse serum, although rabbit and human serums have also been used. The exact composition of these agents is unknown.

a. Hemostatic Serum. This is alleged to be a serum derivative discovered by Dr. Vincent A. Lapenta, composed principally of prothrombin and antiprothrombin in physiological balanced solution. The circulars of the manufacturer make extravagant claims for this alleged hemostatic in "pulmonary hemorrhage, purpura hemorrhagica, intestinal bleeding, bleeding of the newborn, indolent ulcers, and hemorrhages incidental to various surgical procedures such as bone operations, intracranial surgery, herniotomy, tonsillectomy, amputations and hysterectomy." The experimental evidence submitted by Lapenta is uncritical and unsatisfactory.

b. Coagulen. This preparation was inspired by Kocher and Fonio (8) of Berne, and is alleged to be a "physiological styptic prepared from the natural coagulents of animal blood contained in the blood platelets. It has the characteristics of a lipoid." If kephalin is meant, it is difficult to understand why platelets should be selected in preference to other abundantly supplied organs such as brain. The preparation is marketed in three forms: (1) as dry powder containing lactose, (2) as 3 per cent sterile solution in ampoules and (3) tablets. The object of the lactose is stated to be for facilitating solution in water.

Coagulen is recommended for use by practically all known methods of administration and for all kinds of hemorrhages irrespective of cause, origin or etiology, with sweeping generalizations giving the impression of a panacea for bleeding ills. The same may be said of hemostatic serum and coagulose.

c. Coagulose. This is claimed to be an aceton-ether precipitation product of normal blood serum (presumably horse-serum) inspired and originated by G. H. A. Clowes and F. C. Busch (9) and marketed under the name of "Coagulose" by Parke, Davis and Company. "It is a sterile, soluble, anhydrous powder, containing the fibrin ferment necessary for clotting blood. It is readily

soluble in cold water and concentrations two or three times that of original serum concentrations . . . . retaining its active principles unimpaired for long periods of time." The experimental and clinical evidences have been mainly supplied by Clowes and Busch. According to them "Practical experience would indicate that in the large majority of cases of pathological hemorrhage thrombin is not liberated in sufficient quantity or rapidly enough to produce the desired clot." The mechanism of thrombin liberation would depend on the choice of theory of blood coagulation. Howell (10) suggests that an abnormal content of antiprothrombin in hemophilia is possible which should logically require kephalin for treatment rather than a ferment (Coagulose). Nevertheless, Coagulose is advised by the manufacturer's literature, "in all cases of hemorrhage due to defective clotting of blood as seen in hemophilia, hemorrhage of the new-born" and in fact any kind of bleeding no matter what the source, origin or cause, or to be applied locally or systemically. The clinical evidences cited by Clowes and Busch in support of the hemostatic qualities of precipitated blood sera ("Coagulose") are not convincing. The alleged beneficial effects can be accounted for by other factors.

A complete record together with potency guarantees and description of the various thromboplastic preparations tested are presented in table 1.

II. RESULTS

1. Acceleration of coagulation time of oxalate plasma and blood in vitro by various thromboplastic preparations

The methods of testing thromboplastic activity recommended by Howell and a modification of Howell's method by Fenger described in New and Nonofficial Remedies were used with slight modifications. These may be described as follows.

Howell's method. Freshly drawn blood from cat or dog is used. Oxalated plasma and serum are secured. Blood is oxalated so as to contain about 0.1 per cent oxalate. This is then centrifugated and the supernatant portion constitutes oxalated

TABLE 1

Description of thromboplastic agents and claims for potency

NAME	MANUFACTURER	RECORD ON LABEL	DESCRIPTION	POTENCY GUARANTEE	DATE RECEIVED IN LABORATORY
Kephalin (fresh)	Laboratory	February 8, 1919	Light brown		February 8, 1919
Kephalin (old)	Laboratory	May 21, 1918	Deep brown		May 21, 1918
Kephalin (old)	Armour	None	Light brown	None*	May 10, 1917
Kephalin (fresh)	Armour	(H. W. D. February 11, 1919)	Light brown	None	March 4, 1919
Kephalin (sheep)	Laboratory	1912	Black, viscid, tarry		1912
Kephalin (pig)	Laboratory	April 7, 1919	Nearly white, slight yellowish		April 7, 1919
Kephalin	Armour	"The Hemostatic Phosphatid from Spinal Cord and Brain Tissue." From Stevenson, June 6, 1918	Soft, shiny, moist brown waxy masses	None	April 7, 1919
Kephalin	Armour	"Hemostatic Phosphatid from Spinal Cord and Brain Tissue." April 5, 1917	Dry, slight yellowish brown waxy lumps	None	April 7, 1919
Kephalin	Armour	Made April 16, 1917	Soft shiny yellowish brown, moist waxy masses	None	April 7, 1919
Coagulen (dry, old)	Soc. Chem. Industry, Swiss	N/4	Light brown and white mixture powder	None	August 26, 1917

Coagulen (dry, fresh)	Soc. Chem. Industry, Basle	S/6	White powder	None	February 19,1919
Coagulen (liquid)† (fresh)	Soc. Chem. Industry, Basle	Op. No. 9031	Milky white emulsion; 20 cc. large ampoule	None	February 19, 1919
Coagulen (Ciba)	Soc. Chem. Industry, Basle	12118, November 12, 1917	1.5 cc. of 3 per cent solution of coagulen. Small ampoules	None	April 7, 1919
Coagulen Tablets	Soc. Chem. Industry, Basle	Sample K, December 14, 1917	Each tablet—Coagulen Ciba, 0.5 gram, + NaCl = 0.46 gram	None	April 7, 1919
Coagulen—(Kocher Fonio Ciba)	Gesselschaft für Chem. Ind., Basle	1, July 9, 1915	Light brown or yellowish moist powder: discreet brown particles	None	April 7, 1919
Coagulen (Ciba)	Soc. Chem. Industry, Basle	11230, November 12, 1917)	Sterile 20 cc. 3 per cent solution of coagulen, large ampoule	None	April 7, 1919
Hemostatic serum (fresh)	Parke Davis & Company	046461-B	Opalescent fluid with odor of trikresol	August 16, 1920	February 17, 1919
Thromboplastin (fresh)	Squibb	12519	White emulsion with odor of trikresol	August 8, 1920	February 19, 1919
Thromboplastin (old)	Squibb	11041	White emulsion with odor of trikresol	None	April 8, 1917
Thromboplastin (fresh)	Armour	None	White emulsion with odor of trikresol	October 1, 1919	February 17, 1919
Thromboplastin	Squibb, from Stevenson	10441, May 25, 1918	White emulsion with odor of trikresol	December 19, 1918	April 7, 1919
Thromboplastin	Squibb from manufacturer	7661, June 21, 1916	White emulsion with odor of trikresol	June 6, 1917	April 7, 1919

TABLE 1—*Continued*

NAME	MANUFACTURER	RECORD ON LABEL	DESCRIPTION	POTENCY GUARANTEE	DATE RECEIVED IN LABORATORY
Thromboplastin (B)	Armour	None, May 15, 1918	Coarse white emulsion with odor of trikresol	May 14, 1919	April 7, 1919
Thromboplastin (A)	Armour	None, May 15, 1918	Coarse white emulsion with odor of trikresol	May 14, 1919	April 7, 1919
Coagulose	Parke Davis and Company	045098B	White powder	May 20, 1921	May 2, 1919

* None stated.

† "Coagulen-Ciba. 20 cc. in sterile solution ready for use. To be shaken. Op. No. 9681. Importé de Suisse." This was carefully opened April 30, 1919 and contents found to measure 15 cc. Another ampoule with the same label and Op. No. 9641 contained considerable sediment. This preparation is recommended for intravenous injection.

plasma. Blood is drawn into another cylinder and allowed to clot without interference, then centrifugalized for the serum. The control consists of water. The series for testing were made up according to McLean (11) as follows, using small vials about 1.2 cm. in diameter and 4.5 cm. long:

CONTROL	AGENT TO BE TESTED
Oxalated plasma.............. 8 drops Serum........................ 3 drops Normal saline................ 3 drops	Oxalated plasma.............. 8 drops Serum........................ 3 drops Agent, or kephalin (0.1 per cent)........................ 3 drops
Clotting with dog's and cat's blood occurs in from thirty minutes to two hours.	Clotting of same blood used in control occurs in from thirty seconds to two or three minutes

As control, normal saline (0.9 per cent NaCl) was used rather than water as directed by McLean since nearly all of the preparations tested are prepared with saline or recommended to be used in this way. In all of our work complete invertability was used as the end-point of coagulation, and the tests were performed at room temperature.

N. N. R. method. This method was used as described in New and Nonofficial Remedies (12). To 0.5 cc. of thromboplastin solution is added 24.5 cc. normal saline and the mixture shaken until a uniform emulsion results. Of this suspension 5 cc. is mixed in a graduated cylinder with an equal volume of blood serum. To this is added 10 cc. of oxalated beef plasma (containing 0.1 per cent oxalate) and mixed by inverting the cylinder twice. Instead of transferring the mixture to a shallow dish the mixture was left in the mixing cylinder and the whole immersed in a deep water bath kept at 38°C. All solutions were made fresh and saline was used for control. The test of complete invertability was applied throughout.

In the case of preparations limited in quantity the proportions of all constituents were reduced to one-fifth. The dry preparations (kephalin and Coagulen) were used in the same proportions as the liquid thromboplastins, that is, 1 part of the thrombo-

plastic preparation to 100 parts of plasma. This probably gives kephalin a higher concentration but this could not be different owing to the unknown content of kephalin or the active constituent of thromboplastin. The comparison was made on the actual basis of method recommended for testing.

In this series of tests, the plasmas of three different cats, eight dogs and two men were used. Blood of two dogs (experiments 18 and 20) was used as drawn from the femoral artery direct without centrifugalization. About 0.5 cc. of the thromboplastic agent, using an equal volume of saline as control, was added to the blood directly in small vials of uniform size. The mixture was quickly shaken and complete invertability taken as the end-point. The saline controls were made simultaneously with the test for each agent. Experiments were also made with six different beef plasmas.

All of the data obtained are presented in table 2. These represent averages of not less than two and in the majority of instances three trials with each agent.

The results indicate that certain of the agents, namely the thromboplastins and kephalin, though variable, definitely accelerate the coagulation time of both oxalate plasmas and blood as compared with the saline controls. The marked variations and inactivity of certain specimens of these two agents are due to deterioration on standing and will be described in section 3.

Fresh Hemostatic Serum was found to shorten appreciably the coagu'ation time in only four out of eleven experiments, but this was much less than with kephalin and thromboplastin in the same experiments and about the same as the saline controls, indicating the weakness of the product.

Fresh dry Coagulen only moderately shortened the coagulation time in three out of the nine tests made. Fresh Coagulen solution (in ampules) shortened the coagulation time more than saline in only two out of seven tests that were made. Coagulen tablets were not tested since these are prepared from dry Coagulen and would hardly be expected to show anything different.

Coagulose was found to be inactive with bovine and feline plasmas.

In experiments 4 and 6 observations with a miscellaneous lot of agents indicate that acceleration of coagulation time is not limited to kephalin and the thromboplastins. Among these were calcium chloride, fresh saliva and raw and boiled milk. They possessed about one-half to one-third the activity of fresh kephalin under the same conditions. With milk and saliva the effects are to be attributed to calcium and the results are of interest in explaining sources of contamination and possible adulteration (milk). Such colloidal agents as agar, starch, gelatin, acacia, excess of dog serum and also lecithin were without influence. Urine and sulfocyanide were found to prolong the time of coagulation. Apparently thromboplastic activity by kephalin and thromboplastin is not of colloidal nature and certain salts may be detrimental to the process.

Conclusions. The kephalin and thromboplastin type of thromboplastic preparations definitely and rather markedly accelerate the coagulation time of blood and oxalate plasmas in vitro, while Coagulen, Hemostatic Serum and Coagulose are practically inactive.

The coagulation time can also be accelerated by calcium chloride, fresh saliva and milk, while such colloids as starch, gelatin, acacia and lecithin under the conditions appear to be inactive.

2. Acceleration of coagulation time of peptone blood and plasma

Freshly drawn blood from dogs injected with Witte's peptone (1.5 cc. of 20 per cent per kilogram) was used in four experiments and the plasma in one experiment according to Howell's technique, using normal saline as control.

The results in table 3 indicate that clotting of peptone bloods and plasma was also markedly accelerated by the thromboplastic agents. In descending order of efficiency these are thromboplastin, kephalin, and Coagulen. Hemostatic Serum was inactive in the single experiment performed.

TABLE 2

*Acceleration of coagulation time of oxalate plasma and blood in vitro by various thromboplastic agents**

AGENTS AND CONCENTRATIONS USED	NUMBER OF EXPERIMENT																	
	1	2	3*	4†	6‡	8	9	10	10a **	12§	15	18	19	21	22	23	23a	24
	Dog plasma	Dog plasma	Dog plasma	Dog plasma	Dog plasma	Beef plasma	Beef plasma	Beef plasma	Beef plasma	Cat plasma	Beef plasma	Dog blood	Human plasma	Human plasma	Dog blood	Cat plasma	Beef plasma	Cat plasma
	Howell	Howell	Howell	Howell	Howell	N.N.R.	N.N.R.	N.N.R.	N.N.R.	Howell	N.N.R.	Howell	Howell	Howell	Howell	Howell	N.N.R.	Howell
	Coagulation time in minutes																	
Kephalin (sheep, fresh, 2/8/19), 0.1 per cent..	3	4	3	2	7	8	12 (2%)	$3\frac{1}{2}$	$7\frac{2}{3}$	$1\frac{3}{4}$	43	......	......	7	......	......	$3\frac{1}{2}$	$15\frac{1}{2}$
Kephalin (pig's, fresh, 4/7/19), 0.1 per cent ...	......	......	......	......	......	......	......	......	......	$1\frac{1}{12}$	8	$1\frac{1}{3}$	4	$4\frac{7}{10}$	......	......	4	$2\frac{1}{4}$
Kephalin, (Armour, 2/11/19), 0.1 per cent.....	......	6	......	......	......	7	......	$5\frac{1}{2}$	8	1	7	$\frac{17}{30}$ (1%)	$4\frac{3}{4}$	$8\frac{1}{2}$	......	......	2	......
Kephalin (old, 5/21/18), 0.1 per cent........	......	7	......	......	......	7	......	7 (1%)	......	$2\frac{1}{2}$	17	......	......	$8\frac{1}{2}$	......	......	$10\frac{1}{2}$	......
Kephalin (Armour, old, 6/6/18), 0.1 per cent...........	......	......	......	......	......	......	......	......	......	$\frac{57}{60}$	$6\frac{1}{2}$	......	......	$9\frac{1}{4}$	$\frac{11}{30}$	......	$2\frac{1}{2}$	4
Kephalin (Armour, old, 4/5/17), 0.1 per cent)..........	20	......	......	......	......	......	......	......	......	1	8	......	......	$8\frac{1}{2}$	$\frac{11}{30}$	......	$2\frac{1}{2}$	$3\frac{1}{2}$

Kephalin (Armour, old, 4/16/17), 0.1 per cent	......	......	......	......	......	......	......	......	......	1	5	......	......	$9\frac{1}{4}$	$\frac{5}{12}$	......	$2\frac{1}{4}$	$3\frac{1}{2}$
Kephalin (Armour, old, 5/10/17), 0.1 per cent	......	7	......	......	......	25	3 (1%)	5	5	1	7	......	......	$8\frac{1}{2}$	......	......	$2\frac{1}{2}$	......
Kephalin (sheep, old, 1912), 0.1 per cent	......	......	......	......	>35	......	......	>60	......	$4\frac{1}{2}$	......	......	......	......	......	......	>82	$18\frac{1}{2}$
Thromboplastin (Squibb, fresh, 2/19/19), whole	......	3	......	......	......	$\frac{1}{2}$	$\frac{2}{3}$	$\frac{2}{3}$	$\frac{7}{12}$	$\frac{13}{15}$	$\frac{11}{20}$	$\frac{1}{4}$	$1\frac{2}{3}$	$1\frac{1}{2}$	......	......	$1\frac{3}{4}$	2
Thromboplastin (Squibb, old, 5/25/18), whole	......	......	......	......	......	......	......	......	......	$\frac{11}{12}$	25	......	4	$5\frac{1}{2}$	$\frac{1}{2}$	......	10	$2\frac{1}{2}$
Thromboplastin (Squibb, old, 4/8/17), whole	3	4	......	......	......	$\frac{2}{3}$	25	$1\frac{1}{2}$	$2\frac{1}{2}$	$1\frac{1}{4}$	2	......	4	4	......	......	42	......
Thromboplastin (Squibb, old, 6/21/16), whole	......	......	......	......	......	......	......	......	......	$1\frac{5}{6}$	>150	......	12	$5\frac{1}{2}$	1	......	10	......
Thromboplastin (Armour, fresh, 2/18/19), whole	4	4	......	......	......	1	$2\frac{1}{2}$	42	$\frac{13}{15}$	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{5}{12}$	2	$1\frac{1}{2}$	$\frac{1}{4}$	......	2	$1\frac{3}{4}$
Thromboplastin (Armour, old, 5/15/18), whole	......	......	......	......	......	......	......	......	......	1	1	......	10	$4\frac{1}{2}$	......	......	4	$2\frac{1}{2}$

TABLE 2—*Continued*

AGENTS AND CONCENTRATIONS USED	NUMBER OF EXPERIMENT																
	1	2	3*	4†	6‡	8	9	10	10a **	12§	15	18	19	21	22	23	23a	24
	Dog plasma	Dog plasma	Dog plasma	Dog plasma	Dog plasma	Beef plasma	Beef plasma	Beef plasma	Beef plasma	Cat plasma	Beef plasma	Dog blood	Human plasma	Human plasma	Dog blood	Cat plasma	Beef plasma	Cat plasma
	Howell	Howell	Howell	Howell	Howell	N.N.R.	N.N.R.	N.N.R.	N.N.R.	Howell	N.N.R.	Howell	Howell	Howell	Howell	Howell	N.N.R.	Howell
	Coagulation time in minutes																	
Coagulen (dry, fresh, 2/19/19), 3 per cent	$6\frac{1}{2}$ (0.1%)	8 (0.1%)	20 (5%)	......	......	22 (5%)	>204 (1%)	>60	......	4	>300	$1\frac{1}{2}$	21	$8\frac{1}{5}$	......	......	$9\frac{1}{2}$	......
Coagulen (dry, old, 8/26/17), 3 per cent)	......	......	......	......	......	......	......	240	......	$3\frac{1}{2}$	>300	......	12	$8\frac{1}{5}$	......	......	$11\frac{1}{2}$	......
Coagulen (dry, old, 7/9/15), 3 per cent	......	......	......	......	......	......	......	......	......	2	>360	......	12	$8\frac{1}{5}$	$1\frac{1}{5}$	......	$4\frac{1}{2}$	$3\frac{1}{4}$
Coagulen solution (fresh ampoule, 2/19/19), whole	......	38	5	......	......	24	......	......	18 hrs.	......	>300	$\frac{3}{4}$	......	10	......	......	......	......
Coagulen solution (old ampoule, 7/12/17), whole	......	......	......	......	......	......	......	......	......	......	>360	......	$12\frac{1}{2}$	......	......	......	......	......
Coagulen solution (old ampoule, 11/12/17), whole	......	......	......	......	......	......	......	......	......	$3\frac{1}{2}$	>204	......	......	15	......	......	......	......

Hemostatic serum (fresh, 2/17/19), whole..........	1	9	6	......	......	33	......	120	......	$4\frac{1}{4}$	>360	$1\frac{1}{10}$	12	$10\frac{7}{10}$	......	......	14	......
Coagulose (dry, fresh, 5/2/19), 6 per cent.......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	12	7	......
Control (saline)...	25	26	20	12	>180	31	60	240	18 hrs.	$3\frac{1}{2}$	>360	1	$7\frac{1}{6}$	10	$1\frac{2}{3}$	$11\frac{1}{2}$	6	$2\frac{1}{2}$

* The remaining results of this experiment are as follows; fresh saliva, $2\frac{1}{2}$ minutes and 0.3 per cent trikresol, ten minutes.

† The remaining results of this experiment are as follows (figures represent coagulation time in minutes): Fresh saliva $7\frac{1}{2}$, old saliva 10, 0.1 per cent egg lecithin 16, 0.1 per cent agar 20, fresh raw milk 5, fresh boiled milk 6, 5 per cent $CaCl_2$ 4, 0.5 per cent acacia 10, dog serum 25, 0.5 per cent gelatin > 12, 2 per cent starch > 22, 0.1 per cent KSCN > 42, urine > 30.

‡ Additional results in this experiment were: fresh raw milk 15 minutes and fresh boiled milk 16 minutes.

§ A 100 per cent saline extract from fresh dog's liver coagulated this plasma in 4 minutes.

** In experiment 10a the serum of one and the plasma of another animal were used.

‡‡ The concentration of kephalin in plasma by Howell's method is estimated to be about 0.04 per cent; in the N. N. R. method, 1 per cent; also of the thromboplastins. The figures in parenthesis in the first column refer to number of month/day/year, i.e., age of the preparation. The name, "Howell," and the initials, "N.N.R.," in the table refer to the methods used as described in the text. The sign (>) means more than.

TABLE 3

*Acceleration of coagulation time of peptone blood and plasma (dog) by various thromboplastic agents**

AGENTS AND CONCENTRATIONS USED	NUMBER OF EXPERIMENT				
	13	14	16	17	18
	Blood	Plasma	Blood	Blood	Blood
	Coagulation time in minutes				
Kephalin, 0.1 per cent (fresh, sheep, 2/8/19)		>68			
Kephalin, 0.1 per cent (fresh, pig's, 4/7/19)	15		9	$\frac{1}{12}$	Found clotted end of 18 hours
Kephalin, 0.1 per cent (fresh, Armour, 2/11/19)				$\frac{3}{4}$	
Thromboplastin, whole (Squibb, fresh, 2/19/19)	1	$1\frac{1}{2}$		$\frac{1}{10}$	
Thromboplastin, whole (Armour, fresh, 2/17/19)		2		$\frac{1}{4}$	
Coagulen, 3 per cent (fresh, dry, 2/19/19)				$1\frac{1}{2}$	Not clotted end of 18 hours
Hemostatic serum (fresh, 2/17/19)		>60			
Control (saline)	30	>69	16	10	Not clotted end of 18 hours

* Howell's method was used. The sign (>) means more than. The figures in parentheses in the first column refer to dates.

3. *Blood and plasma coagulation efficiency of thromboplastic agents*

In this section only the freshly obtained and prepared agents are considered. The activity of old specimens is discussed in the following section. The detailed data as to the comparative efficiency of the different agents (old and fresh) are presented in table 4, and summarized and grouped in table 5.

Thromboplastins. From the summary in table 5 it is seen that the thromboplastins were distinctly superior to all other products tested, the Squibb product possessing a median efficiency of

more than three times that of the Armour product and seven times that of the freshest and some old kephalins. The greater clotting accelerator activity of the thromboplastins, which are saline or aqueous extracts of tissue, as compared with kephalin, is confirmative of previous observations of Rumpf (13), who found that lipin emulsions (presumably kephalin) prepared from ox brain were far less active than tissue extracts. As compared with the saline control, the fresh thromboplastins hasten coagulation by about ten times with the Armour product and twenty times with Squibb's product.

Kephalin. As compared with the freshest thromboplastins, the freshly prepared and obtained kephalins possessed about one-seventh to one-third the thromboplastic activity. These as well as some old specimens were found to definitely accelerate clotting when compared with the saline control. That is, the coagulation time was only about one-third to one-half as compared with the control.

Serum derivatives. The freshest specimens of dry Coagulen, Coagulen solution in ampules, Coagulose and Hemostatic Serum are seen to occupy the same grouping and efficiency values (table 5) as normal saline. In other words, these products did not accelerate the coagulation of plasmas and blood in vitro, and accordingly are regarded as entirely inert as thromboplastic agents.

Conclusions. The freshly obtained and prepared thromboplastic agents which were tested arrange themselves in descending order of plasma and blood clotting efficiency in vitro as follows: (1) Thromboplastin (Squibb), (2) Thromboplastin (Armour), (3) Kephalins (fresh and some old specimens), (4) Coagulen, Coagulose, Hemostatic Serum and normal saline.

The thromboplastins possess three to seven times the accelerator clotting efficiency of kephalin and shorten the clotting time to one-twentieth to one-tenth as compared with normal saline.

The kephalins (0.1 per cent, fresh and old) possess about one-seventh to one-third the activity of the thromboplastin products, but as compared with saline shorten the coagulation time in vitro to one-third to one-half.

TABI

The plasma and blood coagulation efficien

AGENTS AND CONCENTRATIONS USED	NUMBER C									
	1	2	3	4	6	8	9	10	10a	12
	Order of clotting efficiency and efficiency values (i									
Kephalin (sheep, fresh, 2/8/19), 0.1 per cent	2 (1)	2 (1)	2 (1)	1 (1)	1 (1)	3 (1)	3 (1)	3 (1)	5 (1)	9 (0.62
Kephalin (pig's, fresh, 4/7/19), 0.1 per cent	.....	.....	.....	.....	.....	.....	.....	.....	.....	6 (1)
Kephalin (Armour, 2/11/19), 0.1 per cent	.....	3 (0.66)	.....	.....	.....	3 (0.9)	.....	4 (0.64)	6 (0.9)	5 (1.1
Kephalin (old, 5/21/18), 0.1 per cent	.....	4 (0.5)	.....	.....	.....	3 (0.9)	.....	5 (0.5)	.....	12 (0.4
Kephalin (Armour, old, 6/6/18), 0.1 per cent	.....	.....	.....	.....	.....	.....	.....	.....	.....	4 (1.14
Kephalin (Armour, old, 4/5/17), 0.1 per cent	5 (0.14)	.....	.....	.....	.....	.....	.....	.....	.....	5 (1.1
Kephalin (Armour, old, 4/16/17), 0.1 per cent	.....	.....	.....	.....	.....	.....	.....	.....	.....	5 (1.1
Kephalin (Armour, old, 5/10/17), 0.1 per cent	.....	4 (0.5)	.....	.....	.....	5 (0.33)	2 (4)	4 (0.7)	4 (1.5)	5 (1.1
Kephalin (sheep, old, 1912), 0.1 per cent	.....	.....	.....	.....	>2 (<0.2)	.....	.....	>8. (<0.06)	.....	12 (0.24
Thromboplastin (Squibb, fresh, 2/19/19), whole	.....	1 (1.3)	.....	.....	.....	1 (16)	1 (12)	1 (5.1)	2 (7.6)	1 (1.5
Thromboplastin (Squibb, old, 5/25/18), whole	.....	.....	.....	.....	.....	.....	.....	.....	.....	3 (1.2
Thromboplastin (Squibb, old, 4/8/17), whole	2 (1)	2 (1)	.....	.....	.....	1 (13)	4 (0.5)	2 (2.3)	3 (3)	8 (0.86

arious thromboplastic agents

ERIMENT											MEDIAN ORDER OF CLOTTING EFFICIENCY (1 = MOST EFFICIENT) AND EFFICIENCY VALUE (IN PARENTHESES)
3	14	15	17	18	19	21	22	23	23a	24	
ntheses), when fresh pig's or sheep kephalin (0.1 per cent) = 1											
...	4 (1)	8 (1)	......	......	......	6 (0.67)	......	......	5 (1)	7 (0.14)	3 (1)
2 1)	......	5 (5.4)	3 (1)	5 (1)	3 (1)	4 (1)	......	......	6 (0.9)	2 (1)	4 (1)
...	......	4 (6)	......	3 (1.8)	4 (<1)	9 (0.55)	......	......	2 (1.8)	......	4 (0.9)
...	......	6 (2.4)	......	......	......	9 (0.55)	......	......	12 (0.3)	......	6 (0.5)
...	......	4 (6)	......	......	......	10 (0.5)	2 (0.5)	......	4 (1.4)	6 (0.56)	4 (0.9)
...	......	5 (5.4)	......	......	......	9 (0.55)	2 (0.5)	......	4 (1.4)	5 (0.64)	5 (0.6)
...	......	3 (8.6)	......	......	......	10 (0.5)	3 (0.64)	......	3 (1.6)	5 (0.64)	4 (0.9)
...	......	5 (5.4)	......	......	......	9 (0.55)	......	......	4 (1.4)	......	4 (1)
...	......	......	......	......	......	......	......	......	>16 (<0.04)	7 (0.12)	>8 (<0.2)
1 5)	1 (45)	2 (43)	1 (9.1)	1 (5.3)	1 (2.4)	1 (3.1)	......	......	1 (2)	1 (1.25)	1 (7.6)
...	......	7 (1.7)	......	......	3 (1)	5 (0.85)	4 (0.4)	......	12 (0.35)	3 (0.9)	4 (0.9)
...	......	9 (<0.3)	......	......	3 (1)	2 (1.1)	......	......	14 (0.08)	......	3 (1)

TABLE 4-

AGENTS AND CONCENTRATIONS USED	NUMBER O									
	1	2	3	4	6	8	9	10	10a	12
	Order of clotting efficiency and efficiency values (i									
Thromboplastin (Squibb, old, 6/21/16), whole	......	......	......	......	......	......	......	......	......	10 (0.6
Thromboplastin (Armour, fresh, 2/19/19), whole	3 (0.75)	2 (1)	......	......	......	2 (8)	2 (5)	6 (0.08)	1 (8.7)	2 (1.4
Thromboplastin (Armour, old, 5/18/18), whole	......	......	......	......	......	......	......	......	......	5 (1.1
Coagulen (dry, fresh, 2/19/19), 3 per cent	4 (0.5)	4 (0.5)	6 (0.15)	......	......	4 (0.4)	6 (0.06)	8 (<0.06)	......	13 (0.27
Coagulen (dry, old, 8/26/17), 3 per cent	......	......	......	......	......	......	......	10 (0.015)		13 (0.3
Coagulen (dry, old, 7/9/15), 3 per cent	......	......	......	......	......	......	......	......	......	11 (0.54
Coagulen solution (fresh ampoule, 7/19/19), whole, 3 per cent	......	6 (0.1)	3 (0.6)	......	......	5 (0.33)	......	......	7 (0.4)	......
Coagulen solution (old ampoule, 4/12/17), whole	......	......	......	......	......	......	......	......	......	......
Coagulen solution (old ampoule, 11/12/17), whole	......	......	......	......	......	......	......	......	......	13 (0.3
Hemostatic serum (fresh, 2/17/19), whole	1 (3)	5 (0.44)	4 (0.5)	......	......	6 (0.25)	......	9 (0.03)	......	15 (0.25
Coagulose (dry, fresh, 5/2/19), 6 per cent	......	......	......	......	......	......	......	......	......	......
Control (saline)	5 (0.14)	6 (0.15)	6 (0.15)	7 (0.17)	>3 (<0.4)	6 (0.36)	5 (0.2)	10 (0.015)	7 (0.4)	13 (0.36

* The figures in parentheses in the first column refer to dates. The sign (>) means great

ontinued

XPERIMENT											MEDIAN ORDER OF CLOTTING EFFICIENCY (1 = MOST EFFICIENT) AND EFFICIENCY VALUE (IN PARENTHESES)
13	14	15	17	18	19	21	22	23	23a	24	
arentheses), when fresh pig's or sheep kephalin (0.1 per cent) = 1											
.....	.....	9 (<0.3)	.....	.....	6 (0.2)	5 (0.85)	5 (0.3)	.....	12 (0.35)	.....	8 (0.3)
.....	2 (34)	1 (86)	2 (3.6)	2 (3.2)	2 (2)	1 (3.1)	1 (1)	.....	2 (1.8)	1 (1.3)	2 (about 3)
.....	.....	2 (43)	.....	.....	4 (0.4)	3 (1.04)	.....	.....	6 (0.9)	3 (0.9)	4 (1)
.....	.....	11 <0.14)	4 (0.1)	4 (1.4)	6 (0.3)	8 (0.57)	.....	.....	12 (0.37)	4 (0.7)	6 (0.4)
.....	.....	11 (<0.14)	.....	.....	5 (0.6)	8 (0.57)	.....	.....	12 (0.3)	.....	10 (0.3)
.....	.....	12 (<0.11)	.....	.....	6 (0.3)	8 (0.57)	5 (0.3)	.....	7 (0.8)	.....	8 (0.3)
.....	.....	11 (<0.14)	.....	3 (1.7)	.....	11 (0.47)	.....	.....	.....	.....	6 (0.4)
.....	.....	12 (<0.11)	.....	.....	6 (0.3)	11 (<0.3)	.....	.....	.....	.....	11 (<0.3)
.....	.....	10 (<0.2)	.....	.....	.....	.....	.....	.....	.....	.....	12 (<0.25)
.....	4 (1)	12 (<0.11)	.....	4 (1.2)	6 (0.3)	12 (0.41)	.....	.....	13 (0.25)	.....	6 (0.4)
.....	.....	.....	.....	.....	.....	.....	.....	1	10 (0.5)	.....	Same as normal saline
3 (0.5)	4 (1)	12 (<0.1)	4 (0.1)	4 (1.3)	5 (>0.5)	11 (0.47)	7 (0.2)	1	9 (0.6)	3 (0.9)	6 (0.36)

han; the sign (<), less than.

TABLE 5

Summary of the coagulation efficiency of various thromboplastic agents

GROUP	DESCENDING ORDER OF EFFICIENCY (1=MOST EFFICIENT)	AGENT	BRAND	DATE OBTAINED OR PREPARED†	APPROXIMATE AGE
					months
1. (Strongest)..	1 (7.6)*	Thromboplastin	Squibb, fresh	2/19/19	Fresh
2. (Stronger)...	2 (3)	Thromboplastin	Armour, fresh	2/19/19	Fresh
3. (Strong)...	3 (1)	Kephalin	Sheep, fresh	2/ 8/19	Fresh
	3 (1)	Thromboplastin	Squibb, old	4/18/17	22
	4 (1)	Kephalin	Pig's, fresh	4/ 7/19	Freshest
	4 (1)	Kephalin	Armour, old	5/10/17	21
	4 (1)	Thromboplastin	Armour, old	5/15/18	9
	4 (0.9)	Kephalin	Armour, fresh	2/11/19	Fresh
	4 (0.9)	Kephalin	Armour, old	6/ 6/18	8
	4 (0.9)	Kephalin	Armour, old	4/16/17	22
	4 (0.9)	Thromboplastin	Squibb, old	5/25/18	9
4. (Weak)....	5 (0.6)	Kephalin	Armour, old	4/ 5/17	22
	6 (0.5)	Kephalin	Sheep, old	6/ 6/18	8
5. (Practically inactive)......	6 (0.4)	Coagulen	Ciba; dry,	2/19/19	Fresh
	6 (0.4)	Coagulen solution	Ciba; ampoule	2/19/19	Fresh
	6 (0.4)	Hemostatic serum	Parke, Davis & Company	2/17/19	Fresh
	6 (0.36)	*Control saline;* also Coagulose	Parke, Davis & Company	5/ 2/19	Fresh
6. (Entirely inactive)	8 (0.3)	Thromboplastin	Squibb, old	6/21/16	32
	8 (0.3)	Coagulen	Ciba; dry, old	7/ 9/15	43
	>8 (<0.2)	Kephalin	Old, sheep's	1912	7 years
	10 (0.3)	Coagulen	Ciba; dry, old	8/26/17	18
	11 (<0.3)	Coagulen solution	Ciba; old ampoule	4/12/17	22
	12 (<0.25)	Coagulen solution	Ciba; old ampoule	11/12/17	22

* Numbers in parentheses constitute the median efficiency values. The sign (>) means greater than; the sign (<) less than.

† The figures read number of month/day/year.

Freshly obtained Coagulen, Hemostatic Serum and Coagulose do not hasten coagulation time in vitro and are therefore entirely inert as thromboplastic agents. Accordingly, the potency guarantee of eighteen months for Hemostatic Serum is misleading.

4. Keeping qualities of thromboplastic agents

These may be judged from the summary in table 5 based on results in table 4.

Kephalin—It is seen that the fresh sheep and pig kephalins, made in the laboratory, as compared with the fresh thromboplastins, possessed about one-seventh of the thromboplastic activity of the Squibb specimen and about one-third of that of Armour's, and were about as strong as thromboplastins nine to twenty-two months old.

Three preparations of kephalin (Armour) which had stood for eight, twenty-one and twenty-two months, respectively, were practically as active as two specimens made freshly in the laboratory and also a fresh specimen of Armour's. Two other specimens, one of which was made in the laboratory, and the other obtained from Armour and Company and which were eight and twenty-two months old, respectively, at the time of testing, possessed only about one-half the activity of the freshest kephalins. The oldest kephalin prepared in the laboratory seven years ago was entirely inactive. It is indicated by the results in table 2 that the fresh kephalin prepared from sheep's brain early in February had lost considerable activity by the end of May, i.e., in about 4 months, although with some plasmas and blood its original activity was nearly restored during April and May. Fresh kephalin from pig's brain, which was prepared when it appeared that the sheep kephalin was losing strength, was certainly stronger than the latter, but after standing for about two months it also lost appreciably in activity.

It appears, therefore, that some specimens of kephalin lose their thromboplastic activity gradually on standing, this being demonstrable at the end of about two months. Other specimens that have stood for eight to twenty-two months are somewhat

less active than freshly made specimens, but the difference is practically negligible. Still other specimens eight to twenty-two months old are very much (about one-half) weaker. Very old specimens, such as one of ours that had stood seven years, are very apt to be inert. Deterioration of kephalin is, therefore, a factor that needs to be seriously considered in marketing this product. These results on deterioration of kephalin obtained by us are fairly confirmative of those obtained by Howell and co-workers. What limitation should be placed on the period of activity of kephalin is difficult to state at this time owing to the scarcity of data. This may not be feasible also until more is known about kephalin and the methods of purification. However, it would appear from our results that kephalin, which has stood a year, may have lost considerable activity and should be discarded. Finally, the product should be tested according to some well known method (Howell's or N. N. R.).

Thromboplastins. The fresh specimens were found to be the most active of all the agents tested. Squibb's preparation was over seven times and Armour's about three times as active as kephalin (see table 5). Specimens from both manufacturers which were nine and twenty-two months old possessed only about one-eighth to one-third the activity of the fresh specimens, but about the same activity as kephalins freshly made in the laboratory. A specimen of thromboplastin (Squibb) that was thirty-two months old was entirely inactive. From this it appears that the thromboplastins lose their plasma clotting activity on standing, and to a greater extent than a number of the kephalins studied. As indicated in table 4, six specimens of kephalin, whether freshly prepared or nine to twenty-two months old, were about equally active. However, the kephalins seemed to exhibit greater variability than the thromboplastins. Considering the variability of the individual results, and the fact that with some bloods a very old (twenty-two months') specimen of thromboplastin was about as active as the freshest specimens of both the thromboplastin and kephalin it appears that the potency guarantees of nine months (Armour) and of one year (Squibb) furnished by the manufacturers of thromboplastins is about correct, although specimens nine months old were less active.

Serum products. From the grouping in table 5 it is seen that different specimens of Coagulen, dry, solution in ampoules, both freshly obtained and old; fresh Hemostatic Serum and Coagulose were practically, if not entirely, inactive. These were no more active than the normal saline used as control. The effect of various concentrations (high and low) concerning which data are presented in table 6 did not alter the efficiency position of these agents. In practically every experiment out of the entire series of twenty-one performed, Coagulen, and Hemostatic Serum always occupied the same position as saline. Coagulose was tried out in only two experiments, but the number of tests performed is sufficient to indicate the worthlessness of this product. It is to be noted that the ten different fresh specimens of Hemostatic Serum used possessed potency guarantees until August 16, 1920, that is, about 18 months after delivery by the manufacturer. Experiments with different concentrations to be presently described indicate the total worthlessness of Coagulen, Hemostatic Serum and Coagulose, fresh or old, as clotting accelerators of blood and plasma.

Conclusions. Both the kephalins and thromboplastins lose their thromboplastic activity on standing. This appears to be more variable with kephalin; since some specimens nine to twenty-two months old were as active as the freshest, while other specimens of same age were much less (about one-half) active. Deterioration of kephalin is demonstrable at the end of about 2 months on standing.

Specimens of thromboplastin from different sources 9 to 22 months old possessed about one-eighth to one-third the activity of the freshest specimens from the same sources and the same activity as the freshest kephalin.

An old (thirty-two months) thromboplastin and a very old kephalin (seven years) were found to be entirely inactive.

Fresh or old Coagulen (dry and solution in ampoules), fresh Hemostatic Serum and fresh Coagulose were found to possess no demonstrable thromboplastic activity in vitro.

5. Relation of concentration of thromboplastic agents to the coagulation time of different plasmas

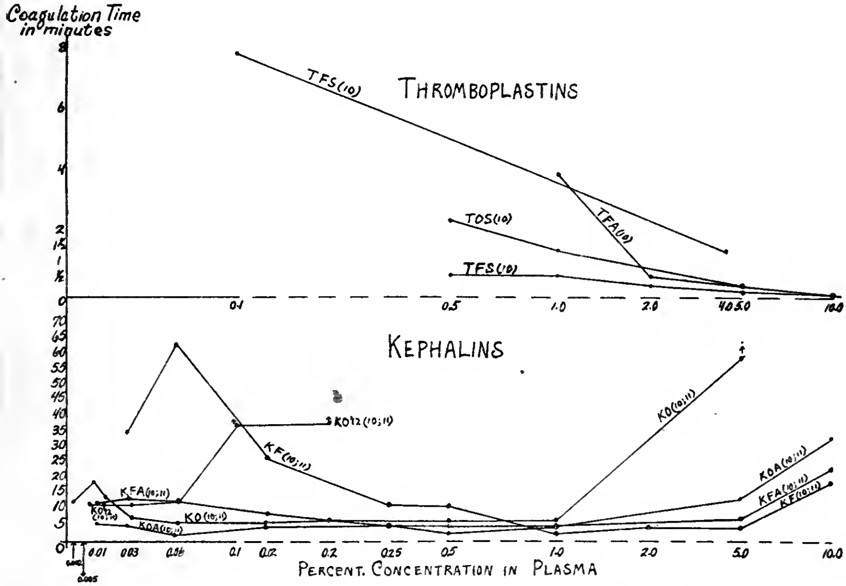
It is conceivable that the results with the less active thromboplastic agents might be different if higher concentrations are used. It will be recalled that only a 0.1 per cent concentration of kephalin was used in table 1, 3 per cent coagulen as a rule, and the thromboplastin extracts and Hemostatic Serum were used undiluted. In testing by Howell's method kephalin is used as a 0.1 per cent solution, the end concentration in the plasma being about 0.04 per cent, while with the use of whole thromboplastins this concentration would be about 37.5 per cent. In the N. N. R., method the end concentration in plasma is about 1 per cent of thromboplastin extract. These concentrations are so different that at least in the case of kephalin, which was used in low concentrations, the differences might be accounted for. However, the discrepancy is more apparent than real, owing to the unknown composition, particularly as to the active constituents, of the thromboplastins, which owing to the poor solubility of kephalin in water, would more nearly approach the weaker than higher concentrations of kephalin emulsions. The results obtained do not sustain this hypothesis.

The matter was tested by observing the coagulation time of different plasmas, using both Howell's and the N. N. R. method, and different concentrations of kephalin ranging from 0.002 per cent to 10 per cent. The concentrations used represent end concentrations in plasma. The results of all the experiments with the different agents tested are presented in table 6, and in the form of curves in the accompanying figure for experiments 10 and 11 with the thromboplastins and kephalins.

These indicate rather strikingly that the behaviour of the kephalins is different from the thromboplastins. With kephalin there appears to be an optimal range of concentration. That is, the greatest acceleration of coagulation is obtained with a range of concentrations from 0.06 per cent to 1 per cent. On either side of these limits, that is, with concentrations lower than 0.06 per cent (down to 0.002 per cent) and higher than 1 per cent

(up to 10 per cent inclusive) the coagulation time is gradually and definitely prolonged. The phenomenon was rather uniform with four different specimens of kephalin, both old and fresh.

On the other hand, the behaviour of the thromboplastins was entirely different. While the acceleration of coagulation by the kephalins was in a sense inversely proportional to the concentration of the phosphatid, the acceleration of coagulation by the thromboplastins was directly proportional to the concen-



THE COAGULATION OF OXALATED BEEF PLASMA ACCORDING TO DIFFERENT CONCENTRATIONS OF THROMBOPLASTINS AND KEPHALINS

The figures in parentheses refer to number of experiment; *F*, fresh; *O*, old; *'12*, 1912; *A*, Armour; *S*, Squibb; *T*, thromboplastins; *K*, kephalin.

tration. The relation is not strictly mathematical, but, in general, it can be said that the higher the concentration the greater the acceleration of coagulation, i.e., a shorter coagulation time with the thromboplastins. Concentrations of the thromboplastins lower than 0.25 per cent were not tried, since lower concentrations gave progressively a longer coagulation time. Therefore, it does not appear incorrect in principle to use relatively higher concentrations of thromboplastin extracts in plasma for

TABLE 6*

The relation of concentration of thromboplastic agents to the coagulation time of different plasmas

NUMBER OF EXPERIMENT	PLASMA USED	METHOD USED	END PER CENT. CONCENTRATION OF THROMBOPLASTIC AGENT IN PLASMA																
			0.002	0.008	0.016	0.032	0.05	0.064	0.1	0.12	0.2	0.25	0.5	1.0	2.0	5.0	10.0	12.5 to 16.0	25
			Kephalin (sheep, fresh, 2/8/19)																
			Coagulation time in minutes																
6	Dog	Howell	....	....	....	....	....	....	7	....	7	....	6	....	....	....	....	....	....
8	Beef	N. N. R.	....	....	....	....	8½	....	8	....	6	....	....	....	....	....	....	....	....
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	3½	5	5	19	....	....
11	Beef	N. N. R.	....	....	....	36	....	64	....	27	....	12½	12	....	....	....	....	....	....
			Kephalin (Armour, fresh, 2/11/19)																
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	5½	....	8	24	....	....
11	Beef	N. N. R.	....	....	....	13½	....	14½	....	9	....	5	5½	....	....	....	....	....	....
			Kephalin (Armour, old, 5/10/17)																
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	5	....	9	34	....	....
11	Beef	N. N. R.	....	....	....	5½	....	5¼	....	3	....	5¼	3	....	....	....	....	....	....
			Kephalin (sheep, old, 5/21/18)																
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	7	....	>60	....	....	....
11	Beef	N. N. R.	12	19	14	7½	....	6½	....	6	7½	....	7	....	....	....	....	....	....
			Kephalin (sheep, old, 1912)																
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	>60	....	....	....	....	....
11	Beef	N. N. R.	....	13	13	13½	....	14	>38	....	>39	>39	....	....	....	....	....	....	....

Thromboplastin (Squibb, fresh, 2/19/19)																			
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	$\frac{2}{3}$	$\frac{2}{3}$	$\frac{1}{3}$	$\frac{1}{4}$	$\frac{1}{6}$	....	......
14	Dog's pepton plasma	Howell	....	....	....	....	....	....	....	....	....	....	....	....	....	1½ (4%)	1 (8%)	1 (16%)	......
21	Human	Howell	....	....	....	....	....	....	8	....	....	....	....	....	....	1½ (4%)	....	....	......
Thromboplastin (Squibb, old, 4/8/17)																			
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	2½	1½	....	$\frac{1}{3}$	....	....	......
Thromboplastin (Armour, fresh, 2/17/19)																			
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	4	$\frac{2}{3}$	$\frac{1}{3}$	$\frac{1}{6}$	....	......
21	Human	Howell	....	....	....	....	....	....	8	....	....	....	4½	....	....	1½ (4%)	....	....	......
Coagulen (fresh, dry, 2/19/19)																			
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	>60	....	....	21	....	......
Hemostatic serum (fresh, 2/17/19)																			
10	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	>120	....	>83	>80	>42 (12.5%)	......
Coagulose (fresh, 5/2/19)																			
23a	Beef	N. N. R.	....	....	....	....	....	....	....	....	....	....	....	7	7	....	6	7 (50%)	6 (25%)
Coagulen (old, dry, 7/9/15)																			
22	Dog blood	Howell	....	....	....	....	....	....	1⅕	....	....	....	60 (3%)	....	....	....	....	....	......

* The sign (>) means more than.

comparison with weaker percentage concentrations of kephalin. In other words, low concentrations (0.1 per cent and even less) of kephalin are entirely adequate for illustrating the maximal acceleration of coagulation power of this agent. This would not necessarily be the case if the kephalin content or the composition of the thromboplastin type of preparations was known. The curves in the accompanying figure illustrating the action of the thromboplastins indicate a different mechanism of action from that of kephalin. This would be contrary to the conception of Howell, according to whom the thromboplastic activity of tissue juice, as represented by the thromboplastic preparations, is due to kephalin, which appears to be a fairly definite chemical entity. Referring to kephalin, Hugh MacLean states "it seems probable that lipins do play some part in the coagulation of the blood, but it does not seem certain that it is the lipins which constitute the thromboplastic substance of the tissues." It is conceivable that the presence of other constituents might alter the behaviour of kephalin in tissue juice (thromboplastin).

The results with Coagulen, Coagulose and Hemostatic Serum, though limited, indicate no uniform activity with either high or low concentrations. They are probably inert in any concentration.

Conclusions. The lag in thromboplastic activity exhibited by kephalin as compared with the thromboplastins is not due to the use of low concentrations.

The range of optimal concentrations of kephalin for hastening the coagulation time of plasma is about 0.06 per cent to 1 per cent. Outside of these limits the coagulation is retarded.

The coagulation accelerator activity of the thromboplastins is directly proportional to the concentration indicating a difference in mechanism of action from kephalin.

Coagulen, Coagulose and Hemostatic Serum, in both high and low concentrations gave, variable results, indicating on the whole the total lack of accelerator thromboplastic activity in vitro of these agents.

III. SUMMARY

1. The kephalin and thromboplastin type of thromboplastic agents definitely and rather markedly accelerate the coagulation time of blood and oxalate plasma in vitro, while Coagulen, Hemostatic Serum and Coagulose are practically inactive.

2. In descending order of thromboplastic activity with pepton plasma the agents tested arrange themselves as follows: thromboplastins, kephalin, Coagulen and Hemostatic Serum (inactive).

3. The freshly obtained or prepared thromboplastic agents tested arrange themselves in descending order of plasma and blood clotting efficiency in vitro as follows: (1) Thromboplastin (Squibb) (2) Thromboplastin (Armour), (3) Kephalins (fresh and some old specimens, Armour, etc.), (4) Coagulen-(Ciba), (5) Coagulose, and Hemostatic Serum (Parke Davis and Company) and normal saline. The thromboplastins possess three to seven times the accelerator clotting efficiency of kephalin and shorten the coagulation to one-twentieth to one-tenth as compared with normal saline. The kephalins (0.1 per cent, fresh and some old) possess about one-seventh to one-third the activity of the thromboplastins, but as compared with saline shorten the coagulation time to one-third to one-half. Fresh Coagulose, Hemostatic Serum and Coagulen did not accelerate clotting. Therefore, these are inert as thromboplastic agents.

4. Both the kephalins and thromboplastins lose their thromboplastic activity on standing. This appears to be more variable with kephalin, since some specimens 9 to 22 months old were as active as the freshest, while other specimens of same age were much less (about one-half) active. Deterioration of kephalin is demonstrable at the end of about two months on standing. Specimens of thromboplastin from different sources nine and twenty-two months old possessed about one-eighth to one-third the activity of the freshest specimens from the same sources and the same activity as the freshest kephalin. An old thromboplastin (thirty-two months) and a very old kephalin (seven years) were entirely inactive. Fresh or old Coagulen (dry or solution in ampoules), fresh Hemostatic Serum and fresh Coagulose possessed no demonstrable thromboplastic activity in vitro.

5. The range of optimal concentrations of kephalin for hastening the coagulation time of plasma is about 0.06 per cent to 1 per cent. Outside of these limits the coagulation is retarded. The coagulation accelerator activity of the thromboplastins (Armour and Squibb) is directly proportional to the concentration indicating a difference in mechanism of action from kephalin. Coagulen, Coagulose and Hemostatic Serum in both high and low concentrations gave variable results, indicating on the whole a total lack of accelerator thromboplastic activity in these agents.

IV. REFERENCES

(1) Hanzlik: J. Pharm. Exp. Therap., 1918, x, 523; 1918, xii, 71; 1918, xii, 119.
(2) Hess: J. Am. Med. Assoc., 1915, lxiv, 1395.
(3) Thudicum, cit. Bang, I: Chemie u. Biochemie der Lipoide, 1911, Wiesbaden.
(4) Howell: Am. J. Physiol., 1912, xiii, 1.
(5) Hammarsten-Hedin: Text Book of Physiological Chemistry, 1915, 7th ed., Wiley & Sons.
(6) MacLean, Hugh: Lecithin and Allied Substances, 1918, Longmans, Green and Company.
(7) Lapenta, V. A.: Therapeutic Gazette, 1918, xxxiv, 23.
(8) Fonio: Corr. Blatt. f. Schweiz, Aerzte, 1913, xliii, 385, 422, 456.
(9) Clowes and Busch: N. Y. Med. J., 1913, January 4.
(10) Howell: Harvey Lectures, 1916–17, Series XII, 273–324.
(11) McLean, J.: Am. J. Physiol., 1916, x' 250.
(12) New and Nonofficial Remedies, 1919, p. 121.
(13) Rumpf, cit. MacLean, Hugh: Loc. cit.

THE HEMOSTATIC PROPERTIES OF THROMBOPLASTIC AGENTS UNDER DIFFERENT CONDITIONS[1]

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I. INTRODUCTION

Practically all of the thromboplastic agents, though differing in origin and composition, are claimed to be specific for or indicated in the treatment of hemophilia, melaena neonatorum, etc. In fact, these remedies are advised for all hemorrhagic tendencies, and also in hemorrhages of normal individuals in whom the tendency to hemostasis has been abundantly met by the natural thromboplastic activity of tissue juice. Under these conditions it appears that the use of thromboplastic agents is irrational and not indicated. This has been pointed out by Professor Howell in his Harvey Lecture of 1917. On the other hand, if it can be shown that a hemorrhagic tendency actually represents deficiency of kephalin, or if the mechanism through

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which kephalin and similar agents act is unfavorably balanced, such, for instance, as an excess of antiprothrombin which, according to Howell (1) may be the case in hemophilia, then kephalin and similar agents would be logically indicated. However, the proof that these disturbances exist in or constitute the etiology of hemophilia does not as yet appear to be sufficiently well established. The scarcity of hemophilia material limits the testing of the thromboplastic agents as hemostatics to ordinary (normal) bleeding. This serves, at least, to test the favorable claims made for these agents in bleeding from surgical wounds of normal individuals.

The ineffectiveness of kephalin and thromboplastin (Squibb) in this direction was indicated in previous studies (2) on quantitative changes in bleeding from superficial wounds of the dog's pad. Owing to the irrigation of most of the pads in these experiments with 1 per cent citrate, which in this concentration has been found (3) to interfere with the activity of kephalin, a final decision concerning its hemostatic qualities could not be made. It was decided, therefore, to extend the experiments, using the agents in saline instead of citrate and under conditions which would be as favorable as possible and yet preserve the quantitative features. To a certain extent the original difficulties have been overcome though not ideally. The wounds under these conditions do not represent bleeding wounds in clinical practice. However, with irrigated wounds it is at least possible to obtain controls of the bleeding from one and the same wound. The bleeding from different wounds even though made to appear alike as possible is variable so that without some kind of controls the results are meaningless. Besides irrigation experiments, observations were also made along clinical lines on hemorrhages from large arteries, liver and bone wounds and on intestinal bleeding in a case of hemophilia. However, the results obtained under all of these conditions were not gratifying. These and the different methods used may now be described. For a description of the agents used and the sources from which these were obtained the preceding paper (4) of this series may be consulted.

II. RESULTS.

1. Effect of thromboplastic agents on superficial hemorrhage from the dog's foot-pad

The favorable results in the preceding paper (4) of this series with kephalin and the thromboplastins on oxalate plasmas and peptone bloods in vitro indicate that these agents might act as hemostatics when applied to wounds irrigated under favorable conditions. That is, in the act of continuous irrigation a constant and sufficient concentration of the thromboplastic agent around the bleeding vessels is assured, while a single application is apt to be washed away or diluted and bleeding will continue, assuming, of course, that the surrounding tissue juice is weak in thromboplastic activity.

The technic of the experiments on dogs was the same as that previously described, except that the irrigations were made with the agents in saline, using normal saline as control. Instead of quantitative estimations of urea-nitrogen or hemoglobin of the wash-fluid, the time of cessation of bleeding (bleeding time) from the denervated foot-pad was used as an index of hemostasis. Wounds of normal animals irrigated with saline cause too much clot formation in the pad wash-fluid rendering colorimetric estimation of hemoglobin unsatisfactory, and the urea content of the agents themselves makes the use of bleeding time preferable. Care was taken to exclude any effects that might be due to changes in systemic blood pressure, which was recorded in the usual way from the carotid artery. The results of the four experiments performed are presented in table 1.

As was anticipated, owing to the variability in bleeding when the pads are irrigated with saline the results are not as satisfactory as when irrigated with citrate. At best a moderate degree of hemostatic activity of the agents tested is indicated. Bleeding stopped promptly when the pads were irrigated with saline alone. In experiment 18, the thromboplastin seemed to possess considerable advantage over saline. In the remaining experiments the results with the different agents are not as striking and might well be within experimental error no matter whether

TABLE 1

Effect of thromboplastic agents on bleeding time from the exposed dog's foot pad

AGENT AND CONCENTRATION USED	AVERAGE BLEEDING TIME	HEMOSTATIC EFFICIENCY VALUE (WHEN KEPHALIN = 1)	DESCENDING ORDER OF HEMOSTATIC EFFICIENCY	REMARKS
Experiment 16 (dog, 8.2 kgm.)				
	minutes			
Saline	16 (2)*	0.44	1. Kephalin	Rate of irrigation 8 to 10 cc. per minute
Kephalin, 0.1 per cent (fresh, pig's)	7¼ (2)	1.0	2. Saline	
Experiment 17 (dog, 6.2 kgm.)				
Saline	3 (4)	0.83	1. Kephalin Saline	Rate of irrigation = 8 to 10 cc. per minute
Kephalin, 0.1 per cent (fresh, pig's)	2½ (2)	1.0		
Experiment 18 (dog, 10 kgm.)				
Saline	16 (4)	0.63	1. Thromboplastin (Squibb)	Irrigation slow; 14 drops in 1 minute
Coagulen, 3 per cent (fresh, 2/19/19)†	>17 (1)	0.6		
Thromboplastin (Squibb, fresh, whole, 2/19/19)	4 (2)	2.5	2. Thromboplastin (Armour)	"
Thromboplastin (Armour, whole, fresh, 2/17/19)	7 (1)	1.4	3. Kephalin (fresh), pig's	"
Coagulen solution, 3 per cent (fresh, 2/19/19)	17½ (1)	0.57	4. Hemostatic serum Kephalin (Armour, fresh) Saline	"
Kephalin, 1 per cent (Armour, fresh, 2/11/19)	15½ (1)	0.64		"
Hemostatic serum (fresh, 2/17/19)	>15 (1)	<0.66	5. Coagulen (dry, fresh) Coagulen solution (ampoule)	"
Kephalin, 1 per cent (fresh, pig's)	10 (1)	1.0		"

Experiment 22 (dog, 12.5 kgm.)

Saline	5½ (2)		1. Kephalin (Armour, 4/16/17)	Slow irrigation; 16 drops in 1 minute
			Thromboplastin (Armour, 5/1/18)	"
Kephalin, 0.1 per cent (Armour, old, 6/6/18)	6 (2)			"
Kephalin, 0.1 per cent (Armour, old, 4/5/17)	5½ (2)		2. Kephalin (Armour, 4/5/17)	"
			Saline	
Kephalin, 0.1 per cent (Armour, old, 2/16/17)	5 (2)		3. Kephalin (Armour, 6/21/18)	"
Coagulen, 0.1 per cent (dry, old, 7/9/15)	7 (2)		4. Coagulen 0.1 per cent (7/9/18)	"
			Thromboplastin (Squibb, 5/25/15)	"
Coagulen (same) 3 per cent	8 (1)			"
Thromboplastin (Armour, old, 5/1/18)	5 (2)		5. Coagulen 3 per cent (7/9/15)	"
Thromboplastin (Squibb, old, 6/21/16)	9 (1)		6. Thromboplastin (Squibb, 6/21/16)	"
Thromboplastin (Squibb, old, 5/25/18)	>7 (1)			

* Figures in parentheses refer to number of observations. The sign (>) means more than; the sign (<) less than.
† These figures in all the tables represent dates on containers; "2/19/19" is to be read February 19, 1919.

the irrigations were made slowly, allowing greater opportunity for clot formation and attachment, or rapidly. The rate of irrigation is not essential, since blood clots formed on wounds even with rapid saline irrigations.

All of the agents used in each experiment have been arranged in the order of descending efficiency, allowing in this way every possible advantage as indicated by the numerical results. In this way, while considerable variability is still apparent, yet, on the whole, the agents arrange themselves in about the same way as the results in vitro indicated in the previous paper (4). That is, the thromboplastins and kephalin are the most active, followed by saline, and finally by coagulen and hemostatic serum as inactive.

In order that small differences in hemostasis, which might not be sufficiently ascertained by this method might be detected, the experiments were altered so as to permit of quantitative observations over longer periods. This was done by rendering the blood noncoagulable with injections of peptone in the animals and collecting the irrigation fluids from the pads for definite periods of time and estimation of hemoglobin in these as best as possible colorimetrically. In the method previously described urea-nitrogen was estimated in the irrigation fluids, but this did not seem feasible with tissue extracts and the serum products. Hemoglobin estimations were made by Palmer's method and were hampered by cloudiness from kephalin principally. The remaining agents did not seriously interfere. The value of 100 per cent was assigned to the first control irrigation fluid and the remaining irrigations were compared with this, representing, therefore, relative percentage changes in bleeding.

The results obtained and presented in table 2 are not ideal, but indicate in a general way the tendency exhibited by the agents in unpeptonized animals and in vitro. In two out of three experiments kephalin was found to possess a greater hemostatic efficiency than in the saline controls. In the most satisfactory experiment performed (experiment 17) thromboplastin (Armour) was superior to all the agents tried and coagulen seemed to behave about like thromboplastin. The tendency exhibited by the

TABLE 2

Effect of thromboplastic agents on superficial hemorrhage from the foot pads of peptonized dogs

AGENT AND CONCENTRATION USED	DURATION OF IRRIGATION	CHANGE IN HEMORRHAGE (+ = INCREASE; − = DECREASE)	HEMOSTATIC EFFICIENCY VALUE (WHEN KEPHALIN = 1)	DESCENDING ORDER OF HEMOSTATIC EFFICIENCY	REMARKS
Experiment 13 (dog, 7.2 kgm.)					
	minutes	*per cent*			
Saline	10	−21	About 2.0		
Saline	20	−53			
Kephalin, 0.1 per cent (fresh, sheep)	12½	−11	About 1.0	1. Saline	
Saline	17	−22.5	About 1.12	2. Kephalin	
Saline	28½	−30			
Kephalin, 0.1 per cent (fresh, sheep)	15½	−20	About 1.0		
Experiment 16 (dog, 8.2 kgm., peptonized)					
Saline	10	−30	About 1.0		Irrigation rate = 8 to 10 cc. per minute
Saline	44½	−30	<0.5	1. Kephalin	
Kephalin, 0.1 per cent (fresh, pig's)	20	−58	1.0	2. Saline	
Experiment 17 (dog, 6.2 kgm.)					
Saline	36	+142	About 0.3		Irrigation rate = 8 to 10 cc. per minute
Kephalin, 0.1 per cent (fresh, pig's)	40	−45	1.0	1. Thromboplastin (Armour) Coagulen	
Saline	6½	−28	>0.6		"
Saline	11½	No change	0	2. Kephalin	"
Thromboplastin (Armour, fresh, 2/17/19)	17	First + 62; Later −100	2.2	3. Saline	"
Saline	5	−64	>1.4		"
Coagulen, 3 per cent (fresh, dry, 2/19/19)	10½	−100	>2.2		"

* The various signs have meanings as follows: (>) = more than; (<) = less than.

results of this experiment confirms the results of experiments 18, 16, 17 and to some extent experiment 22 in which unpeptonized animals and saline irrigations were used.

Conclusions. The hemostatic effects of the thromboplastic agents tested on superficial hemorrhage from the dog's pad, although limited and variable, in general tend to agree with their power to accelerate coagulation of blood and oxalate plasma in vitro.

Accordingly, the thromboplastins and kephalin are among the most active, followed by saline and coagulen and hemostatic serum as either inactive or doubtful. However, these results do not necessarily furnish the indication or rationale for usage in clinical practice.

2. Effect of various thromboplastic agents on hemorrhage from femoral arteries

To meet the objection that irrigation wounds do not meet the requirements of clinical conditions the use of a method practiced by Hirschfelder (5) was resorted to. By this method Hirschfelder reported favorable hemostatic effects from kephalin in arterial bleeding. The idea appears to be that the dissected and exposed Scarpa's Triangle offers a favorable site for clot formation and attachment which would not be the case with wounds exhibiting a free flow of blood, or with irrigations.

The method consists in exposing the femoral arteries in the Scarpa's Triangles of both extremities of the dog by dissection, leaving an excavated recess in which the blood will collect and the clot attach itself to the walls. One artery is painted with kephalin or the thromoplastic agent and the other is left untreated. In our experiments this artery was always flooded with saline as control. The arteries are then transsected and the coagulation time and bleeding time observed, taking note of the blood lost by siphoning into graduated vessels. According to Hirschfelder the blood from the artery painted with kephalin exhibits a shorter coagulation time and bleeding time and the quantity of blood lost is less than from the untreated artery.

All told 16 experiments were made on dogs and cats.

Thirteen experiments were made with kephalin, the thromboplastins (Armour and Squibb), coagulen and hemostatic serum, and three without the use of any agents whatever.

The thromboplastic agents were dissolved or suspended in saline and applied to one artery and an equal quantity of saline to the other artery. The escaping blood was collected in tared shallow pans placed under the gluteal regions, using a partition of paste board in the median line in order to prevent mixing of blood from the two wounds. The initial blood pressure was recorded from the carotid artery of each animal, and previously lowered in some, since it was believed that low levels of pressure would offer more favorable conditions for clotting, therefore, hemostasis. In other respects the technique was carried out in the same way as described by Hirschfelder.

As would be expected it was impossible to obtain wounds which were exactly alike. Furthermore, shreds of tissue are apt to predominate in one wound more than in the other and one artery is apt to retract more than the other thereby facilitating hemostasis. If the severed end of the blood vessel happens to be covered by a pledget or fold of tissue this also tends to stop bleeding. These factors, it is believed, will account for the great variability in results obtained with treated and untreated vessels. As a rule, the animals did not struggle, but in dogs there was considerable spurting of blood when the blood pressure was high, rendering accurate collections of blood impossible. Despite these defects the method was used and the agents tested owing to the claims that have been made for kephalin. The results are presented in table 3.

Kephalin. In two animals with low blood pressures and treated with concentrations of 2 per cent and 50 per cent of fresh kephalin the coagulation time and bleeding time, as far as could be judged, were about the same from both vessels. The coagulation time of the blood of these animals, which were peptonized, was markedly accelerated by kephalin in vitro (see previous paper (4)). In another animal the bleeding from the artery treated with saline stopped sooner and the loss of blood was about one-sixth of that from the kephalinated vessel.

TABLE 3

Effect of various thromboplastic agents on hemorrhage from femoral arteries in Scarpa's triangles

AGENT AND CONTROL USED	QUANTITY AND CONCENTRATION USED	FEMORAL ARTERY TO WHICH APPLICATION WAS MADE	COAGULATION TIME	BLEEDING TIME	QUANTITY OF BLOOD LOST	LEVEL OF SYSTEMIC BLOOD PRESSURE AT TIME OF APPLICATION OF AGENTS
Experiment 16 (dog, 8.2 kgm., peptonized)						
	cc.		*minutes*	*minutes*	*grams*	*mm.Hg.*
Kephalin (fresh, pig's)	5.0 (2%)	Left	3	3		26
Normal saline.*	5.0	Right	3	3		
Experiment 17 (dog, 6.2 kgm., peptonized)						
Kephalin (fresh, pig's)	5.0 (50%)	Right	11	11	65.0	60
Normal saline	5.0	Left	7	7	66.0	
Experiment 33 (dog, 10 kgm.)						
Kephalin (Armour, fresh, 2/11/19)	5.0 (50%)	Left	5	>5	237.0	110
Normal saline	5.0	Right	2	5	41.0	
Experiment 20 (dog, 18 kgm.)						
Thromboplastin (Armour, fresh, 2/17/19)	0.5 (whole)	Left	3	3	75.0	15
Normal saline	0.5	Right	3	3	50.0	
Experiment 22 (dog, 12.5 kgm.)						
Thromboplastin (Squibb, fresh, 2/17/19)	0.5 (whole)	Left	$\frac{1}{4}$	$\frac{1}{2}$	11.5	104
Normal saline	0.5	Right	$4\frac{1}{2}$	6	267.2	
Experiment 26 (cat, 616 grams)						
Thromboplastin (Squibb, fresh, 2/19/19)	0.5 (whole)	Left	$3\frac{1}{4}$	$5\frac{3}{4}$	12.1	140
Normal saline	0.5	Right	$3\frac{1}{4}$	$5\frac{3}{4}$	3.0	
Experiment 32 (dog, 7 kgm.)						
Thromboplastin (Armour, fresh, 2/17/19)	1.0 (whole)	Left	$2\frac{1}{2}$	5	67.0	120
Normal saline	1.0	Right	5	5	411.0	

TABLE 3—*Continued*

AGENT AND CONTROL USED	QUANTITY AND CONCENTRATION USED	FEMORAL ARTERY TO WHICH APPLICATION WAS MADE	COAGULATION TIME	BLEEDING TIME	QUANTITY OF BLOOD LOST	LEVEL OF SYSTEMIC BLOOD PRESSURE AT TIME OF APPLICATION OF AGENTS
Experiment 35 (dog, 15 kgm.)						
	cc.		*minutes*	*minutes*	*grams*	*mm.Hg.*
Thromboplastin (Armour, fresh, 2/17/19)	5.0 (whole)	Left	½ to 2	2	47.0	112
Normal saline	5.0	Right	3	3	681.0	
Experiment 36 (dog, 13 kgm.)						
Thromboplastin (Armour, fresh, 2/17/19)	5.0 (whole)	Right	2	75	831.0	120
Normal saline	5.0	Left	1	2	387.0	
Experiment 27 (cat, 635 grams)						
Coagulen (fresh, dry, 2/19/19)	0.5 (3%)	Left	2	2½	5.0	110
Normal saline	0.5	Right	2	2½	12.6	
Experiment 34 (dog, 12 kgm.)						
Coagulen (dry, fresh, 2/19/19)	5.0 (5%)	Left	1	2½	267.0	100
Normal saline	5.0	Right	1	2½	273.0	
Experiment 28 (cat, 740 grams)						
Hemostatic serum (fresh, 2/17/19)	0.5 (whole)	Left	2¾	5¼	17.8	110
Normal saline	0.5	Right	2¾	5¼	13.3	
Experiment 37 (dog, 5 kg.)						
Hemostatic serum (fresh, 2/17/19	2.5 (whole)	Right	3	3	231.0	120
Normal saline	2.5	Left	1	1	43.0	
Experiment 29 (dog, 7 kgm.)						
None	None	Left	17	17	117.0	80
None	None	Right	2½	3	150.0	
Experiment 30 (dog, 10 kgm.)						
None	None	Left	3½	1	247.0	163
None	None	Right	3½	1	50.0	
Experiment 31 (dog, 12 kgm.)						
None	None	Left	5	2	337.0	120
None	None	Right	5	2	221.0	

* 0.9 per cent NaCl. The sign (>) means more than.

Thromboplastin. Five dogs and 1 cat with blood pressures ranging from 15 to 140 mm. were treated with fresh thromboplastins (Armour and Squibb), using different quantities. The coagulation time and bleeding time in three animals (experiments 20, 26 and 35) were practically unchanged. In another animal (experiment 36) the coagulation time and bleeding time were markedly shortened by saline. In only one (experiment 22) out of six animals were the coagulation time and bleeding time from the vessel treated with thromboplastin markedly shortened and the loss of blood was much less than from the control. In two other animals (experiments 32 and 35) the coagulation time was shortened somewhat, the bleeding time being unaltered and the loss of blood was less than from the control arteries. Therefore, it would appear that 50 per cent of the animals whose arteries were treated with thromboplastins yielded results more or less favorable to the hemostatic action of these agents.

However, this is doubtful when bleeding from animals whose vessels were not treated at all (experiments 29, 30 and 31) is considered, particularly as to loss of blood which was different from the two blood vessels of each animal. In two of these animals the coagulation time and bleeding time were the same, but in one animal (experiment 29) the coagulation time and bleeding time from one vessel were about one-sixth of that of the other and the loss of blood was greater. In other words it appears that what favorable results were obtained with the thromboplastins were accidental owing to the tremendous range of experimental error present in the method. Low levels of blood pressure did not seem to offer any advantage over high levels.

Serum products. So far as bleeding time and coagulation time are concerned these were the same in the four animals (experiments 27, 34, 28 and 37) treated with coagulen and hemostatic serum, but the loss of blood from the arteries in two of the animals was extremely variable. Magalhaes (6) claims hemostatic action for a hemostatic serum in tubal abortion and cesarean section, but whether this is the same preparation as used by us is not known.

The lack of uniformity in the results and the undecisiveness of the method prompted us to abandon further experimentation along this line. Moreover, it is doubtful if any advantage can be secured by the addition of tissue extracts or their active constituents (such as kephalin) to such large wounds already bountifully supplied with tissue juice.

Conclusions. The extreme variability of results obtained after the application of various thromboplastic agents to dissected femoral arteries bleeding into Scarpa's Triangles and the results from untreated vessels under similar conditions indicate that this method of testing these agents is unreliable and unsatisfactory.

If anything, the results indicate the worthlessness of kephalin, coagulen, and hemostatic serum and probably also of the thromboplastins as hemostatics under these conditions.

3. *Effect of various thromboplastic agents on hemorrhage from bone and liver wounds*

Several tests with the different thromboplastic agents were made on a bone wound in the right tibia of a dog, and also on stab wounds of different depths in the liver. The bone wound consisted of an excavation exposing the marrow and was about 1 cm. wide and 4 cm. long. A slow oozing of blood was obtained. Cessation of bleeding and coagulation were observed by direct inspection. In the majority of tests the agents were applied by saturating a cotton pledget with which the wound was gently swabbed once every minute until the bleeding stopped. At times irrigations were also practiced. Saline was used as the control. The results are presented in table 4, and indicate no important differences as compared with the controls.

With the bone wounds, bleeding generally tended to continue irrespective of the agent applied; also true of the controls with saline. This is in accordance with the general reputation enjoyed by bone wounds in surgical practice. The clotting of blood, which took place, seemed inadequate to stop the oozing of blood.

TABLE 4

Effect of various thromboplastic agents on hemorrhage from bone and liver wounds. (experiment 25; dog, 14 kgm., blood pressure level = 60 mm.)

Bone wound (excavation in right tibia)

CONTROLS, AGENTS AND CONCENTRATION USED	MODE OF APPLICATION	COAGULATION TIME	BLEEDING TIME
		minutes	*minutes*
Normal saline*	Direct irrigation	2 (soft clot)	2
Thromboplastin; whole (Armour, fresh, 2/17/19)	Direct irrigation; 0.5 cc.	$2\frac{1}{4}$	$2\frac{1}{4}$ (more blood in wound)
Normal saline	Direct irrigation; 0.5 cc.	2 (soft clot)	2
Thromboplastin; whole (Armour, fresh, 2/17/19)	Direct irrigation; 0.5 cc.	$>3\frac{1}{2}$	$>3\frac{1}{2}$ (bleeding unchecked)
Normal saline	Wiped with cotton pledget every 60 seconds until bleeding ceased		>6 (bleeding unchecked)
Thromboplastin; whole (Armour; fresh, 2/17/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	$>2\frac{3}{4}$ (soft clot)	>8 (bleeding unchecked)
Normal saline	Wiped with cotton pledget every 60 seconds until bleeding ceased	3	>8 (bleeding unchecked)
Kephalin, 5 per cent (fresh, pig's, 2/19/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	$3\frac{3}{4}$ (soft clot)	8 (bleeding unchecked)
Normal saline	Wiped with cotton pledget every 60 seconds until bleeding ceased	2	>8 (bleeding unchecked)

TABLE 4—*Continued*

CONTROLS, AGENTS AND CONCENTRATIONS USED	MODE OF APPLICATION	COAGULATION TIME	BLEEDING TIME
		minutes	*minutes*
Coagulen, 3 per cent (fresh, dry, 2/19/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	5	>$8\frac{1}{2}$ (bleeding unchecked)
Hemostatic serum (fresh, 2/17/19)	Wiped with cotton pledget every 60 seconds until bleeding ceased	9	>9 (bleeding unchecked)
Epinephrin 1:1000	Wiped with cotton pledget every 60 seconds until bleeding ceased	$4\frac{1}{2}$	>9 (bleeding unchecked)

Liver wounds (wiped once every minute with cotton pledget saturated with agent

CONTROLS, AGENTS AND CONCENTRATIONS USED	DEPTH OF STAB	BLEEDING TIME
	cm.	*minutes*
Normal saline	Moderate 0.5	$\frac{3}{4}$
Normal saline	0.5	$1\frac{1}{6}$
Thromboplastin, whole (Armour, fresh, 2/17/19)	0.5	$1\frac{1}{4}$
Normal saline	1.0	3
Thromboplastin, whole (Armour, fresh, 2/17/19)	1.0	$1\frac{1}{2}$
Normal saline	1.0	$1\frac{1}{2}$
Kephalin, 5 per cent (fresh, pig's, 2/7/19)	1.0	47 seconds
Normal saline	1.0	$1\frac{1}{10}$
Kephalin, 5 per cent (fresh, pig's, 4/7/19)	1.0	$2\frac{1}{6}$
Normal saline	1.0	$1\frac{1}{4}$
Kephalin, 5 per cent (fresh, pig's, 4/7/19)	1.0	$4\frac{1}{6}$
Normal saline	1.0	$1\frac{1}{2}$
Coagulen, 3 per cent (fresh, dry, 2/19/19)	1.0	$1\frac{1}{6}$
Coagulen, 3 per cent (fresh, dry, 2/19/19)	1.0	$2\frac{1}{3}$
Hemostatic serum (fresh, 2/17/19)	1.0	$1\frac{2}{15}$
Hemostatic serum (fresh, 2/17/19)	1.0	$3\frac{1}{12}$
Epinephrin 1:1000	1.0	$3\frac{1}{12}$
Epinephrin 1:1000	1.0	$2\frac{1}{12}$
Normal saline	1.0	$5\frac{7}{12}$

* Normal saline = 0.9 per cent NaCl. The sign (>) means more than.

The bleeding from stab wounds of the liver, both shallow and deep, stopped promptly, whether treated with saline alone or the thromboplastic agents. The results did not warrant further extension of similar experiments, which are difficult to control properly and are, therefore, unsatisfactory as pointed out in a previous publication by one of us (P. J. H.).

Conclusions. The applications of the various thromboplastic agents to bone and liver wounds of a dog, gave unsatisfactory results as to hemostasis. If anything these were negative and do not support the claims that have been made.

4. The effect of systemic hemorrhage on the coagulation time of blood

The various thromboplastic agents and preparations on the market are recommended for use by almost every known method of administration, including the intravenous, for both local and systemic bleeding. Howell (1) has reported favorable effects on blood coagulation after the gastric administration of kephalin to dogs. That is, coagulation time was accelerated, but it was also observed by Howell that bleeding itself tends to hasten coagulation. It was our original intention to test the various thromboplastic agents along this line, but the results obtained with bleeding alone were so discouraging that the attempt was abandoned.

The method consisted of withdrawing blood from the femoral artery, of a morphinized dog into small vials and observing the coagulation time using complete invertability as the end point.

The results presented in table 5 indicate that bleeding alone markedly and rather promptly shortened the bleeding time in the four animals that were observed.

Taking the experiments individually it is seen that coagulation time was hastened by 75 per cent in fifty-four minutes, being unchanged at the end of one hour and four minutes after blood was first drawn in experiment 16. In experiment 17, coagulation time was shortened by 92 per cent in thirty-eight minutes, 90 per cent in forty minutes and about 67 per cent at the end of

forty-seven minutes after bleeding was started. In experiment 18, coagulation time was shortened by 75 per cent in forty minutes and 70 per cent at the end of fifty-three minutes after bleeding was started. In experiment 22 the coagulation time was shortened by 78 per cent at the end of ten minutes, 90 per cent at the end

TABLE 5*

Effect of hemorrhage on the coagulation time of blood in dogs

NUMBER OF EXPERIMENT	CONDITION	NUMBER OF BLOOD SAMPLE							
		1	2	3	4	5	6	7	8
		Coagulation time end of minutes after bleeding started							
13	Normal, 10:20.........	10							
	Peptonized, 11:10......	>33 (80)	14 (69)	16 (78)	>347 (347)				
16	Normal, 10:06.........	8	$7\frac{1}{2}$ (8)	2 (54)	2 (64)				
	Peptonized, 11:26$\frac{1}{2}$.....	2 (96)	4 (97)	9 (108)	9 (116)	7 (144)			
17	Normal, 9:58..........	9	$\frac{3}{4}$ (38)	$\frac{5}{6}$ (40)	3 (47)				
	Peptonized, 10:58......	27 (88)	75 (116)	80 (177)					
18	Normal, 10:10.........	3	$2\frac{1}{2}$ (16)	3 (22)	$\frac{3}{4}$ (40)	$\frac{3}{4}$ (46)	$\frac{5}{6}$ (53)		
	Peptonized, 3:01.......	>1080 (23 hrs.)							
22	Normal, 10:08.........	8	$1\frac{3}{4}$ (10)	$1\frac{1}{2}$ (41)	$\frac{5}{6}$ (56)	$1\frac{1}{2}$ (86)	$1\frac{2}{3}$ (102)	$2\frac{1}{3}$ (113)	$2\frac{1}{4}$ (140)

* Figures enclosed by parentheses represent number of minutes after withdrawal of blood was begun. The left femoral artery was used in all experiments. The sign (>) means more than.

of fifty-six minutes and 72 per cent at end of one hundred and forty minutes after the blood was first drawn. From this it appears that any favorable claims which may be made for the systemic administration of thromboplastic or other agents on the coagulation time of blood obtained by repeated bleedings at

intervals from an artery must be regarded with suspicion. We considered that the investigation of thromboplastic agents along this line was entirely precluded on the basis of our results obtained with normal and untreated animals.

Some of the animals were later peptonized with the idea of obtaining a non-coagulable blood which could be drawn over considerable periods of time without the natural tendency to hasten clotting time from bleeding alone. The blood of such animals was temporarily rendered non-coagulable although variable in the four animals (table 5) that were tried, but all of these suffered a fatal fall of blood pressure and died shortly after the injection of peptone.

Such experiments could hardly be expected to show any more than peptonized plasma or blood in vitro, data concerning which are presented in the first paper (4) of this series.

Conclusion. Bleeding at intervals from arteries of normal animals markedly (by 72 to 90 per cent) and promptly (ten to forty-seven minutes) shortens the coagulation time of blood, rendering it impossible to arrive at any definite conclusion concerning the thromboplastic activity of agents administered systemically.

5. *Thromboplastic agents in hemophilia*

Logically it appears that kephalin and similar thromboplastic agents are not therapeutically indicated in any condition, including hemophilia, unless the tissues are deficient in the thromboplastic agent, or the blood contains an excess of some of the constituents such as antithrombin which interferes with clotting and on which kephalin is considered to act. According to Howell (1), an excess of antiprothrombin in hemophilia is considered possible. Neutralization of this by kephalin should accelerate the coagulation time, and, therefore, furnish a rational therapeutic remedy. Favorable results are reported to have been observed by Howell (1), also by Lucas and Hurwitz (7), and others. Lowenburg and Rubenstone (9) observed acceleration of clotting of hemophilic blood with thromboplastin

(Squibb). According to others (8) the etiology of hemophilia is different; and equally good or bad hemostatic results are reported by the use of the agents based on an entirely different conception of blood coagulation. Thus it appears that the inharmonious views and contradictory reports on this subject render any conclusions insecure and dubious. This is due in part to the relatively few cases of hemophilia available for definite observations, the impossibility of securing reliable controls, the uncontrolled clinical observations and enthusiastic reception accorded to any new remedy. These tendencies are too prevalent in the few reports in the literature on the therapeutics of hemophilia. The literature of the manufacturers of this class of preparations generally credits the thromboplastic agents with specificity in hemophilia and other bleeding conditions.

During the course of our work there was an opportunity to administer kephalin to a patient with hemophilia. Although it might be inferred that the use of kephalin in this patient was beneficial, it is not believed that such a conclusion is justified, since bleeding stopped spontaneously on occasions before the administration of kephalin and conceivably might have stopped again in spite of the administration of kephalin during the long attack of intestinal bleeding. The abbreviated protocol of the patient follows:

H. H., boy; age, fourteen years.

Past history. Sick three months; came into Lakeside Hospital with hemarthria; previously known to be hemophiliac. Bled recently as a result of tonsillectomy. Bled profusely all night from tonsils. There were hemorrhages also in knee joints. Sustained abrasion of elbow joint in operating room and bled continuously for several days. Suture of abrasion was resorted to and finally bleeding stopped spontaneously. Boy was transfused at the time of severe tonsillar hemorrhage. Bleeding from transfusion incision was difficult to stop, but eventually ceased. Prolonged coagulation time and bleeding time were demonstrated in the hospital. Diagnosis, hemophilia by Professor C. F. Hoover.

Present illness. On February 25, 1919, the boy was at Rainbow Cottage with bleeding from bowels. Fresh macroscopic blood was

present in stools, which were rather liquid for the last eight days. Blood was continuously present; benzidin was positive and blood cells were present microscopically. Boy was very anemic. The following medication was given by Dr. F. Blankenhorn, using fresh sheep kephalin prepared in the laboratory and which markedly accelerated clotting of blood and oxalate plasma.

February 26. 0.5 gram kephalin per os 7.00 a.m.
0.5 gram kephalin per os 7.00 p.m.
February 27. 1 gram kephalin same as on February 26.
February 28. 1 gram kephalin same as on February 26.
March 1. 1 gram kephalin at 7.30 a.m.
Bleeding time (right ear)[2] = 21 minutes.
Coagulation time (right ear) = 18 minutes.
Administration of kephalin stopped. Total given 4 grams.
March 2. Stool claimed to be free from blood.
March 3. Absence of blood in stool confirmed.
March 4. No blood in stool microscopically.
March 5. Benzidine test positive; no microscopic blood in stool.
March 6. No blood in stool microscopically (two smears).
March 8. No blood in stool microscopically.
March 9. No blood in stool microscopically.
Right ear. Bleeding time = 9 minutes. Clotting time = 14 minutes.
Left ear. Bleeding time = 11 minutes. Clotting time = 16 minutes.
During the last four months the patient has remained well.

Conclusions. The administration of 4 grams of kephalin by mouth to a patient with hemophilia and suffering with a troublesome intestinal hemorrhage was followed by prompt stoppage of the hemorrhage and shortening of the coagulation time and bleeding time of ear blood. This is not interpreted as due necessarily to kephalin, since bleeding in this patient was known to have stopped spontaneously before the treatment, illustrating the necessity of exercising the greatest care in interpreting data of this sort.

[2] Duke's (10) methods for obtaining coagulation time and bleeding time were used. The normal range of coagulation time according to Duke is from five to seven minutes; of bleeding time, one to three minutes. The results obtained indicate definite prolongation of both the bleeding time and coagulation time. The patient was so anemic that the withdrawal of larger quantities of blood and venupuncture necessary in the Morawitz method were precluded.

III. SUMMARY

(The numbers refer to the sections in the text)

1. The hemostatic effects of the thromboplastic agents tested on superficial hemorrhage from the dog's pad, although limited and variable, in general tend to agree with their power to accelerate coagulation of blood and plasma in vitro. Accordingly the thromboplastins and kephalin are among the most active; followed by saline and coagulen and hemostatic serum as either inactive or doubtful.

2. The extreme variability of results obtained after the application of various thromboplastic agents to dissected femoral arteries bleeding into Scarpa's triangles and the results from untreated vessels under similar conditions indicate that this method of testing these agents is unreliable and unsatisfactory. If anything, the results indicate the worthlessness of kephalin, coagulen, hemostatic serum and probably also the thromboplastins, as hemostatics under these conditions.

3. The application of various thromboplastic agents to bone and liver wounds of a dog gave unsatisfactory results. If anything, these were negative and do not support the claims that have been made for them.

4. Hemorrhage per se at intervals from arteries of dogs markedly (by 72 to 90 per cent) and promptly (ten to forty-seven minutes) shortens the coagulation time of blood, rendering it impossible to arrive at any definite conclusions concerning the thromboplastic activity of agents administered systemically.

5. The administration of 4 grams of kephalin by mouth to a patient with hemophilia and suffering with a troublesome intestinal hemorrhage was followed by prompt stoppage of the bleeding with shortening of the coagulation time and bleeding time of ear blood. This is not interpreted as due necessarily to kephalin, since bleeding in this patient was known to have stopped spontaneously before the treatment, illustrating the necessity of exercising the greatest care in interpreting data of this sort.

IV. REFERENCES

(1) Howell: Harvey Lectures, 1916–1917, Series XII, pp. 273–324.
(2) Hanzlik: J. Pharm. Exp. Therap., 1918, xii, 71 and 1918, xii, 119.
(3) Waksman: Am. J. Physiol., 1918, xlvi, 375.
(4) Hanzlik and Weidenthal: J. Pharm. Exp. Therap., 1919, 14:81.
(5) Hirschfelder: Lancet 1915, ii, 542; Berl. klin. Wochschn. 1915, lii, 976.
(6) Magalhaes: Arch. Brasil de Med., 1918, viii, 735.
(7) Hurwitz and Lucas: Arch. Int. Med., 1916, xvii, 543.
(8) Minot and Lee: Arch. Int. Med., 1916, xviii, 474; Hess: Arch. Int. Med., 1916, 17: 203. Ducceschi: Arch. ital. biol., lxiv, 341.
(9) Lowenburg and Rubenstone: J. Am. Med. Assoc., 1918, lxxi, 1196.
(10) Duke, W. W.: J. Am. Med. Assoc., 1910, lv, 1185.

BENZYLCARBINOL: A LOCAL ANESTHETIC

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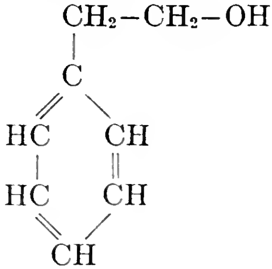
INTRODUCTION

In general, cocaine substitutes have been constructed on the benzoic acid nucleus as esters. There is, however, one exception in the case of peronin (benzylmorphine), which is a benzyl ether. This substance has anesthetic properties (1), but it was not until Macht (2) discovered that benzyl alcohol manifested similar powers that the action could be ascribed to the benzyl grouping. From the preceding it is evident that benzyl alcohol, and its ethers are possessed of anesthetic powers as well as the benzoic acid esters. It is, however, a question whether this property can be attributed to the above groupings alone, or whether it is common to other alcohols, ethers and esters of aromatic side-chain compounds. With this point in mind the present study of the anesthetic properties of benzylcarbinol was made, and compared with phenmethylol and procaine.

CHEMICAL AND PHYSICAL PROPERTIES

Benzylcarbinol or β-phenylethylol, also known as rose oil or orange oil, has the following structural formula:



[1] This research has been supported by a grant from the Committee on Scientific Research of the American Medical Association.

It is a volatile oil with a rose-like odor. Its boiling point is 220°C., and specific gravity 1.0235 at 15°C. In nature it occurs in the volatile oils of roses, orange flowers, and pine needles. It is optically inactive. The solubility of the benzylcarbinol we have found to be about (slightly under) 2 per cent in water, and the aqueous solutions may be sterilized (in autoclave) without diminishing its pharmacological activity.

HISTORICAL

Blondel (3) gives the following history of the medicinal uses of rose preparations: Curative properties were attributed to roses by physicians as far back as Hippocrates. Dioscorides claimed that roses were equally effective in all diseases known to pathology at that time. Of especial interest in the present connection was its use by him in collyria. The medical preparations mentioned by Dioscorides, which owe their potency to the perfume of flowers, include oil of roses. This substance was not the same as essence of roses, but was prepared by macerating the petals in olive oil. Amongst these preparations are also included troches of roses, which the Roman women carried around their necks. Galen, Pliny and the Arabs continued the use of the rose preparations as described by Dioscorides. The ancients attributed to it the following properties: stimulant, tonic, stomachic, and aphrodisiac.

THERAPEUTIC USES

At the time Blondel wrote his paper (1) essence of rose was used for its stimulant and antispasmodic properties, a quality in common with other substances of strong odor, such as musk and asafoetida. Essence of orange had the same properties. The essence of rose was prescribed as (1) an astringent (a property which is doubtful), (2) as an anodyne, and (3) as a nerve tonic.

TOXICITY AND PHARMACOLOGICAL ACTION OF ESSENCE OF ROSE

According to Blondel the essence of rose when ingested by a fasting individual for ten days, starting with five drops and increasing this amount each day by one drop, causes a marked cramp of the stomach. When two drops on a piece of sugar are ingested the same result is obtained. When taken by mouth at the end of meals it appeared to stimulate the digestive functions of the stomach for the first few days. If taken with an initial quantity of ten to twelve drops, the cramps in the stomach reappear, and the digestive functions are not stimulated, but on the contrary are hindered. Flatulence was also noted. Aside from the above the only other effect observed was a marked tendency toward sleep.

The same author injected 1 cc. of the essence of rose subcutaneously in a guinea-pig on four successive days, at the end of which time the animal died, after a torpor of two days duration. The only pathological change revealed on autopsy was a slight meningeal congestion. Prolonged exposure to the vapors of the essence caused no untoward effects as shown by reports of manufacturers of the perfume. He also exposed himself to the vapors for days and nights without suffering any ill effects. The boiling vapors cause some irritation of the eyelids and a slight headache.

INVESTIGATIONS WITH ROSE OIL

Pure rose oil has not hitherto been subject to pharmacological investigation.

Toxicity of benzylcarbinol

The minimal lethal dose was determined by us in white mice of 20 to 30 grams weight. When injected subcutaneously in emulsions in physiological saline solution it was found to be 0.041 cc. or 0.041 gram. It was administered in the form of an emulsion to avoid the injection of too large a volume.

In the case of one mouse a general anesthesia of about an hour's duration was observed, after which the animal moved

about voluntarily and gradually became normal. In other cases there were muscular incoördination and weakness. Exophthalmos was also observed. The mice which died were in a state of coma for several hours before death. Respiratory changes were not constant. There were no signs of irritation at the site of injection.

In a dog (9 kgm. brown fox terrier), 1.83 grams of benzylcarbinol were administered intravenously, in the form of a 3 per cent emulsion in physiological saline solution. The pulse rate was doubled in a few minutes, but soon became normal; the pupils were markedly enlarged, but reacted to light; and, extreme salivation was observed. After the injection the dog passed through three stages: (1) depression, in which there was no attempt at moving when urged, (2) attempts at walking, with muscular incoördination, and (3) excitement, where the animal ran about the room, aimlessly. Several hours after the injection retching movements were noticed, but these disappeared in about two hours more. Recovery was rapid, no effects being evident after twelve hours had elapsed.

From the above record it appears that benzylcarbinol is a relatively harmless substance, possessing about the same order of toxicity as benzyl alcohol.

Anesthetic effect

a. Anesthesia on the tongue. When a drop of pure rose oil is placed on the tongue a burning sensation is felt, which is followed by anesthesia of the exposed area. There is no distinguishable difference between the anesthesia thus produced and that of benzyl alcohol.

b. Anesthesia of the rabbit's cornea. In these experiments a determination of the minimal concentration of rose oil which would anesthetize the rabbit's cornea was made. Parallel experiments were made with benzyl alcohol.

The technique consisted of instilling in the conjunctival sac about 0.5 cc. of the physiological saline solution of the substances studied, and then observing the time at which the anesthesia

was obtained, and when it began to disappear. The results are included in table 1.

TABLE 1*

Benzylcarbinol and phenmethylol on rabbit's cornea

SOLUTION	BENZYL CARBINOL			BENZYL ALCOHOL		
	Result	Onset	Duration	Result	Onset	Duration
per cent		*minutes*	*minutes*		*minutes*	*minutes*
0.5	Good	1	3			
	Nil					
	Nil					
	Nil					
0.75	Light	$1\frac{1}{2}$	$1\frac{1}{2}$			
	Nil					
	Good	1	3			
	Good	1	5			
	Good	2	5			
	Very light					
	Very light					
1.0	Light	2	2	Light	$1\frac{1}{2}$	$2\frac{1}{2}$
	Light	1	2	Light	$1\frac{3}{4}$	$2\frac{1}{2}$
	Good	1	4	Very light	1	4
	Good	1	4	Nil		
	Good	1	$2\frac{1}{2}$	Light	2	$2\frac{1}{2}$
1.25				Good	$1\frac{1}{2}$	$5\frac{1}{2}$
				Good	1	10
1.5				Good	$1\frac{1}{2}$	$2\frac{1}{2}$
				Good	1	4
2.0	Good	1	10			
	Good	1	13			

* Under "results": "Good" means anesthesia to pressure by a probe; "Light," anesthesia to light pressure; "Very light," to bare touch; "Nil," failure of anesthesia.

It is evident from the above record that rose oil in 1 per cent solution is as efficient an anesthetic upon the rabbit's cornea as 1.25 per cent benzyl alcohol.

Up to the present only a few experiments on the human eye have been made; these were carried on with the aid of Dr. E. M. Blake. A light anesthesia was produced in several cases but the rose oil by itself is not free from irritating effects.

c. *Anesthetic effect upon the human skin as determined by the wheal method* (4). In the experiments here included, the minimal concentration of rose oil in physiological saline solution required to anesthetize the skin to light touch was compared with solutions of benzyl alcohol and of procaine.

The results of these experiments are shown in table 2. In each wheal 0.5 cc. of the anesthetic solutions were injected. In each of the three subjects three wheals were made across the volar surface of the upper part of the forearm for each concentration and experiment. The instrument used for touching the wheals consisted of a broom straw tipped with cotton.

Table 2 shows that rose oil[2] anesthetized the skin in thirteen cases out of twenty-one in 1/40 per cent solution, whereas procaine hydrochloride was effective six times in twelve, and benzyl alcohol only once in nine cases. The average time of onset of the anesthesia was about the same in all three compounds. The duration of the anesthesia was a little in favor of the rose oil.

The minimal concentration of benzyl alcohol effective for anesthesia by the wheal method was found to be 1/30 per cent by Hjort and Kaufmann (5). One experiment above checks that work. Sollmann (6) found the concentration of procaine,

[2] The rose oil used was the commercial product of the Fries and Fries Company. The benzyl alcohol was made in this laboratory by the action of sodium hydroxide on benzaldehyde, and purified by several distillations from an ordinary distilling bulb, and finally from a Lebel-Henninger distillation tube. This product was very pure when freshly distilled, for it was entirely soluble in 1 per cent solution, a fact worthy of mention, for in a few days, even if kept in dark bottles, slow oxidation results in the production of material which is insoluble in water. This insoluble fraction in the course of a year amounts to about 40 per cent of the original pure products. The experiments were carried on over a period of several weeks after the distillation of the benzyl alcohol, consequently the small amount of decomposition may to some extent affect the results. The procaine hydrochloride was kindly contributed by Dr. T. B. Johnson, having been made in his laboratory. In every case the solutions were made freshly on the day of the experiment.

TABLE 2*

Effect of benzylcarbinol, phenmethylol and procaine in dilute concentratipns injected intradermally

SOLUTION	SUBJECT		BENZYLCARBINOL (β-PHENYLETHYLOL)			PHENMETHYLOL (BENZYL ALCOHOL)			PROCAINE-HCl		
			Wheal number			Wheal number			Wheal number		
			1	2	3	1	2	3	1	2	2
per cent											
1/30	S	o.	—	3.0	1.0				—	5.0	2.5
		d.	—	3.0	4.0				—	2.0	2.5
	H	o.				3.0	3.0	3.0			
		d.				4.0	4.0	4.0			
	E	o.							5.0	5.0	5.0
		d.							2.0	2.0	2.0
1/40	E	o.	4.0	4.0	—	—	—	—	—	—	1.5
		d.	19.0	19.0	—	—	—	—	—	—	2.0
	S	o.	—	3.0	—	—	—	—	—	—	3.0
		d.	—	10.0	—	—	—	—	—	—	1.5
	H	o.	—	2.0	1.5	—	2.5	—			
		d.	—	6.0	5.5	—	5.0	—			
	E	o.	—	—	3.0				u.	3.0	2.0
		d.	—	—	2.0				3.0	4.5	4.0
	S	o.	—	4.5	3.0				—	—	3.0
		d.	—	3.0	3.0				—	—	u5.0
	H	o.	5.0	2.5	2.0						
		d.	6.5	6.0	5.5						
	E	o.	—	3.0	2.5						
		d.	—	4.0	4.0						
1/50	E	o.	—	—	—						
		d.	—	—	—						
	S	o.	—	—	—						
		d.	—	—	—						
	H	o.	—	—	2.5						
		d.	—	—	3.5						

* In this table: o., indicates onset of anesthesia; d., duration; —, no anesthetic effect; and u., unsatisfactory.

which produces similar results, to be 1/32 per cent. The above is a corroboration of his work.

From these observations it seems that the local anesthetic power of rose oil is a little superior to that of procaine and benzyl alcohol, as determined by the wheal method.

One of the present authors injected 1 cc. of a 1 per cent solution of the benzylcarbinol subcutaneously in the volar surface of the forearm, which became anesthetic to needle pricking for a period of five minutes. Only a momentary sting was felt at the time of injection, after which complete numbing of the area resulted.

DISCUSSION

In comparing the relative merits of the anesthetics herein studied the rose oil seems to be somewhat superior to the other two. It is cheaper, less toxic, more stable, and of little better anesthetic power than procaine. As compared with benzyl alcohol it is of about the same toxicity, somewhat more potent as an anesthetic both on the cornea and in the skin, more stable, but not quite as soluble. The solubility of the rose oil, however, falls within the range required for therapeutic use. The benzylcarbinol is a commercial product found on the market regularly, for it is used in the perfume trade.

It is apparent from the work recorded in the foregoing pages that not only the benzoic acid esters, the benzyl ethers, and benzyl alcohol have local anesthetic powers, but at least one other aromatic side-chain alcohol as well. This paper contains only one of the latter type of compounds, but other closely related ones are being prepared for similar investigation.

Further clinical studies are being planned.

SUMMARY

1. Rose oil, another aromatic side-chain alcohol, possesses local anesthetic properties which from laboratory studies seem to be superior to those of benzyl alcohol.

2. The toxicity of rose oil as determined on white mice and a dog is about the same as that recorded by Macht for benzyl alcohol.

3. Benzylcarbinol is more stable than benzyl alcohol, another point in favor of the former.

4. The solubility of the rose oil is sufficient for its therapeutic use.

The authors wish to thank Mr. Ephraim Shorr for acting as a subject in some of the experiments.

REFERENCES

(1) Buffalini, Guaita and Pierard: Quoted by Guathmey in his Textbook of Anesthetics, New York, 1914.
(2) Macht, D. I.: Journ. Pharm. and Exp. Therap., 1918, xi, 263.
(3) Blondel, R. E.: Thesis. Les Produits Odorants des Rosiers., Paris, 1889.
(4) Hoffmann and Kochmann: Beit. Klin. Chir., 1914, xci, p. 489.
(5) Hjort and Kaufmann: Work to be published later.
(6) Sollmann, T.: Journ. Pharm. & Exp. Therap., 1918, xi, 69.

THE COMPARATIVE SKIN IRRITANT PROPERTIES OF DICHLORETHYLSULPHIDE ("MUSTARD GAS") AND OTHER AGENTS[1]

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WITH THE ASSISTANCE OF

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The extensive use of chemical substances in gas warfare led to a systematic study of compounds which irritate the skin. The various compounds submitted by the Chemical Offense Section of the Chemical Warfare Service were compared with dichlorethylsulphide ("mustard gas"), imitating field conditions in warfare as closely as possible. That is, the skin irritant efficiency of the new compound as compared with dichlorethylsulphide was determined. A brief summary of the methods used and results obtained is here presented. This may be of interest in toxicology and pharmacology, particularly since it is believed that about one-half of the 70 compounds studied have not been previously described.

METHODS

Direct application. This was made use of principally in chronic experiments with various animals. The method was devised by Lynch at the American University. A 5 sq. cm. area of skin suitably prepared (by shaving and washing) was selected

[1] Published by permission of the Director, Chemical Warfare Service, U. S. A.

and to this was applied 0.005 cc. (from a pipette graduated to deliver 0.005 to 0.05 cc.) or 5 mgm. of the compound equivalent to the application of 0.001 cc. (or 1 mgm.) per square centimeter of skin. No precaution was taken to confine the vapors of volatile compounds. The time of appearance of hyperemia and other changes was noted, and the extension and severity of all changes was studied quantitatively as much as possible from day to day and the results expressed in terms of square centimeters of area involved. Dichlorethylsulphide was chosen as the standard by which the irritant properties of other compounds were judged.

On human skin the tests were made by touching a small area with a fine glass rod dipped into the irritant. This method could not be used quantitatively.

Vapor. Preliminary tests with vapors of volatile compounds were made by two methods devised by Lynch. One method consisted of placing a small excess of the compound on a plug of cotton in a test tube of 1 cm. diameter and 5 cm. from the opening of the tube. This was allowed to stand long enough to secure a saturation of the column of atmosphere in the tube and then the tube was applied for variable lengths of time to the skin.

The other method consisted of placing a small excess of the compound on a plug of cotton in the bottom of a test-tube (1 cm. diameter and about 10 cm. long) which was enclosed in a water-jacket made from a long test-tube (20 by 180 mm.). The tube was stoppered and allowed to stand for one hour at about 25°C. before exposure to the skin was made. The vapor concentration in both methods was unknown although in the latter it was practically saturated. For quantitative purposes the following method was used and in connection with it a special skin applicator was devised.

Quantitative vapor method.[2] This consisted of blowing air by means of an ordinary motor blower through concentrated sulphuric acid and calcium chloride, then through a bubbler, containing

[2] Since the compilation of this report, a paper by Smith, Clowes and Marshall (J. Pharm. Exp. Therap., 1919, xiii, 1) describes essentially the same method.

the compound, and connected with a series of glass skin applicators by means of glass tubing as much as possible. The skin applicator consisted of a small glass cylinder of about 1.5 to 2 cm. in diameter and about 4 cm. long with a small glass handle attached on top, 2 small tubes at opposite ends for the passage of air laden with the vapors of the compound, and an opening on the bottom for the exposure of skin to the vapor. This opening was 1 cm. in diameter, and remained closed until it was established that the vapors were of constant concentration. When the concentration of vapor was constant, the exposure to the skin was made directly for any desired length of time.

The concentration of vapor was determined by dividing the loss of weight of the compound in the bubbler by the total volume of air, which was passed through as indicated by the flowmeter. The concentration (nominal) was expressed in terms of milligrams per liter. With certain compounds, the nominal concentrations agreed within 10 to 20 per cent by chemical analysis and this was satisfactory enough for our purposes. The skin irritant efficiency of the vapors of different compounds was judged by comparison of the lowest effective concentrations and the per cent of positive responses to approximately equal concentrations of the vapors, using dichlorethylsulphide as standard.

This method may be suitable for the study of anesthetics and other volatile agents on skin. A photograph of the apparatus is presented in the accompanying figure.

Use of compounds in solution. The compounds were dissolved in suitable solvents such as absolute alcohol, liquid petrolatum, olive oil, benzene, carbon tetrachloride, chlorbenzol, etc., for the purpose of determining the lowest effective concentrations, and for the determination of their skin irritant efficiencies, using 2 or 3 solutions which were not effective to 4 or 5 which showed positive effects. A definite quantity, usually about 0.02 cc., was applied to a definite area (1 sq. cm.) of human skin. Larger quantities, that is, about 0.1 cc. per 5 sq. cm. of skin, were used on dog's skin, so that the concentration per square centimeter of skin was exactly the same for the different species.

However, it was found that so far as the determination of skin irritant efficiency for our purposes was concerned the use of the compound in different solvents was not always satisfactory. With certain compounds the skin irritant efficiency determined in this way did not agree with those determined by direct appli-

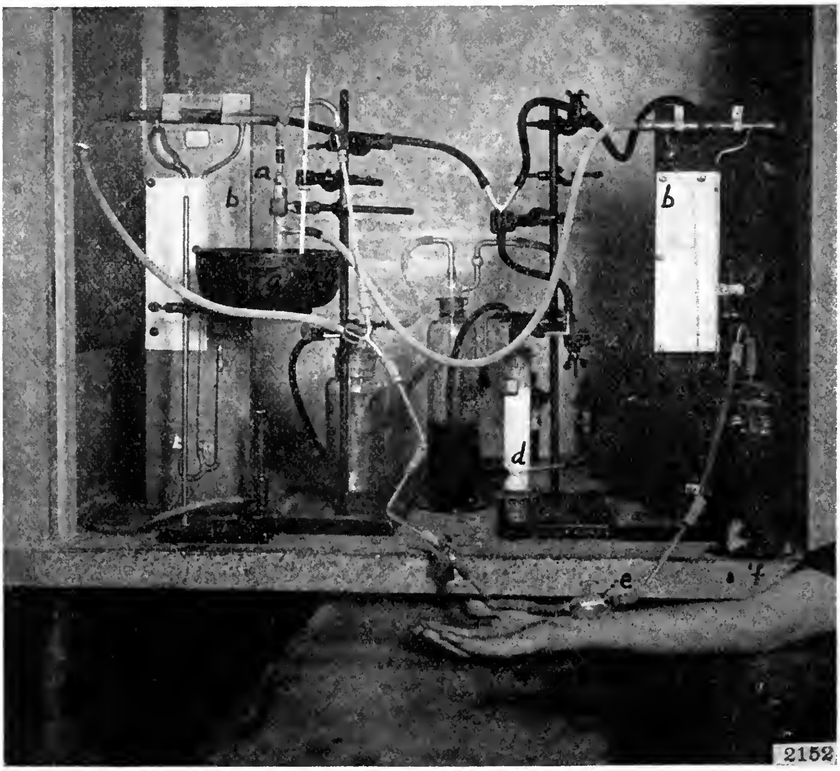

FIG. 1. APPARATUS FOR QUANTITATIVE VAPOR METHOD

a, bubbler, containing compound; *b*, flow meter; *c*, concentrated sulphuric acid; *d*, calcium chloride; *e*, skin applicator; *f*, tower for absorbing vapors of the compound, containing charcoal, permanganate and soda-lime; *g*, water-bath.

cation and the different vapor concentrations. This is attributed to differences in volatility, lipoid solubility, coefficient of solubility of compound in skin and solvent, formation of insoluble and decomposition products, etc. Under the conditions these factors could not be adequately investigated.

Selection of species. Dogs were used principally owing to the limited amount of human material and the large number of compounds studied. Human skin was used as much as permissible and possible. Monkeys, a horse and the cock's comb and wattles were also used. The skin of monkeys responds in the same way as that of dogs. The cock's comb responded principally by local inflammation and necrosis.

So far as we know the skin of animals does not blister. Mild effects of irritants on the skin of animals are indicated by simple hyperemia, rashes, moderate swelling and edema, also moderate petechial hemorrhages. Instead of vesication, the more severe effects are characterized by rather extensive edema and swelling, and the severest effects by gangrene and ulceration.

On the other hand, the acute and more severe effects on human skin are characterized by hyperemia and vesication, sometimes pustulation and the severest effects also by ulceration. The scars in the skins of different species do not differ greatly, and depend upon the degree of ulceration and extent of destruction of tissue. The skin of the Mexican or African hairless dog is said to respond to the action of such irritants as cantharides in the same way as human skin, but this was not tried. What relation the sudoriporous and sebaceous glands played in the entry (absorption) of the compounds into skin was also not studied. In this connection it is interesting to note that horse's skin, which contains sweat glands, was found to be more sensitive to a number of the compounds than the skin of men, dogs and monkeys. This observation is confirmative of the French.

Rodents were not used in our work, but the results obtained by others indicate that their response to the various irritants is about the same as that of dog's skin.

No marked differences between shaved and unshaved skin of men were observed, greater susceptibility in animals perhaps being in favor of shaved skin. Great individual variability in both human and animal skins was encountered. It appears that repeated exposure of human skin renders it sensitive and eventually unreliable for further experimentation, although the original lesions may be completely healed. As a rule, pigmentation of

skin tended to protect against the irritation by the different compounds. This was frequently tested out in animals by exposing a portion of a large freckle or pigmented spot with neighboring white skin in animals simultaneously to the irritants (vapors and liquid) and almost invariably the pigmented area escaped from the effects entirely. It was suggested by Professor Sollmann in Cleveland and observed by Captain E. K. Marshall at American University that the skin of negroes was more resistant than the skin of whites to irritant effects of dichlorethylsulphide ("mustard gas").

SUMMARY OF RESULTS

The following brief summary compiled from a large number of results may be presented at this time:

1. The following compounds were found to be severe irritants as indicated by hyperemia, swelling and edema, ulceration, necrosis, etc., on dog's skin and similar changes together with vesication on human skin; arsenic trichloride, bromine trifluoride, chlorisonitrosoacetone, dinitrochlorbenzol (parazol), ethyldichlorarsine, dichlorethylsulphide ("mustard gas"), iodine pentafluoride, methyldichlorarsine, methyl and propyl "mustard" mixture, methyldibromarsine, phenyldichlorarsine, selenium bromine ethylene derivative, dichlordiethylselenide, "mustard" titanium tetrachloride and di-isothiocyandimethylether.

2. The following compounds were found to be mild irritants as indicated by simple hyperemia without vesication, mild urticarial rash, moderate swelling and edema and very little or no necrosis; normal butyldichlorarsine, O-chlor-chloracetanilide, chloracetophenone, oil from chloracetophenone, chlorethylmethylsulphide, Costa Rica tree sap,[3] dimethylarsinecyanide, diphenylchlorarsine, diphenylcyanarsine, dichlordiethyltellinochloride, dichlordimethyldithioloxalate, iodoacetophenone, isothiocyanmethylether, isothiocyandimethylether, monochlorethylacetate, monobromethylacetate, selenium chlorine ethylene derivative, selenium ethylene bromine compound, selenium acetylene chlo-

[3] Composition unknown.

rine compound, dichlordivinyl selenide, trichlordiethylselenide, selenium "mustard," trimethylthioarsenite, trimethylarsenite and chlorphenarsazene.

3. The following compounds produced no objective or subjective symptoms on human and dog's skin; ammonia silicon tetrafluoride, bromacetamide, benzyl sulfocyanate, ethyl ester of fluorsulphonic acid, "mustard"-mercuric chloride product, juglon, lead tetra-methyl, lead tetraphenyl, mercury dimethyl, methyl "mustard," parabromchloracetophenone, tetrachlordinitroethane and mercury trichlorethylene.

4. As a rule the active arsenicals acted more severely than dichlorethylsulphide during the acute stages. The lesions were more painful, indurated and attached. The ulcers were sharply punched out, clean, dry and possessed red bases. Healing occurred promptly. The differences between the different arsenicals were principally quantitative.

5. Dichlorethylsulphide acted more slowly than the arsenicals. The acute effects were less pronounced, and it was more chronic. There was less destruction of tissue in the beginning. Swelling and edema were marked, pouchy and soft in animals. The ulcers were irregular, dirty, purulent and foul. The lesions were generally painless, and secondary infection was common. Healing was slow.

6. As judged by the clinical effects in chronic experiments, lowest effective concentrations of the compounds in solution, and in vapor form, the skin irritant efficiency of the more important severe irritants was about as follows in descending order of efficiency: dichlorethylsulphide ("mustard gas"), phenyldichlorarsine and methyldichlorarsine. By direct application, dibromarsine and selenium compounds come next in order.

7. The order of protein precipitant power of some of the arsenicals tested agrees in the same direction with their skin irritant efficiency, and it was possible to correlate this in a general way with the quality of skin lesions produced. On the other hand, dichlorethylsulphide ("mustard gas") was the most efficient skin irritant, and its power to precipitate protein was almost negligible, indicating a difference in mechanism of action.

8. Regarding pigmentation of the skin after healing of lesions of several different compounds, the following characteristic features were encountered:

Absence of pigment—dichlordinitrosoacetone.

Faint brown pigment—dichlorethylsulphide.

Deep brown pigment—arsenicals.

Metallic grey pigment—organic selenides.

9. These and other differences in the behavior of a number of the compounds that were noted indicate differences in mechanism of action dependent on differences in chemical structure and composition of the irritants and various physical-chemical and physiological factors. These offer possibilities for further elucidation and correlation of the relations between chemical structure and pharmacological action.

Other possibilities worthy of consideration are the uses of these compounds in the production of experimental lesions, and in therapeutics (arsenicals in syphilis, etc.).

ANAPHYLACTOID PHENOMENA FROM THROMBOPLASTIC AGENTS[1]

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The thromboplastic agents which have been discussed in preceding papers (1) may be placed into two groups according to the absence or presence of precipitable protein. The results of the tests for protein are presented in table 1, and the following tabulation is based on these.

Protein absent	*Protein present*
Kephalin (fresh; prepared in laboratory)	Hemostatic serum (Parke Davis Company)
Coagulen (Ciba)	Thromboplastin (Squibb)
Coagulen solution (Ciba)	Thromboplastin (Armour)
Kephalin (Armour)	

Accordingly, those preparations containing protein might be expected to produce symptoms resembling anaphylaxis when injected intravenously. The literature of the manufacturers gives the contrary impression, but Lee and Minot (2) have stated that such products as Coagulen, etc., may be dangerous when injected.

The proposition was tested by making intravenous and subcutaneous injections into healthy unsensitized guinea-pigs, using saline, and trikresol (0.3 per cent), which is used as a common preservative for one of these agents, as controls. The intravenous injections were made slowly into either the left or right jugular veins in animals previously lightly etherized for incision of the skin only. The symptoms after injection were observed and

[1] This research was supported in part by a grant from the Therapeutic Research Committee, Council of Pharmacy and Chemistry of the American Medical Association.

TABLE 1

*Precipitation of protein by heat and salting**

HEMOSTATIC SERUM	THROMBOPLASTIN (FRESH, SQUIBB)	THROMBOPLASTIN (FRESH, ARMOUR)	KEPHALIN (FRESH, SHEEP 0.1 PER CENT)	COAGULEN (DRY, FRESH) 5 PER CENT	COAGULEN SOLUTION 3 PER CENT (FROM AMPOULE)	KEPHALIN (OLD, SHEEP) 0.1 PER CENT	KEPHALIN (FRESH. ARMOUR) 0.1 PER CENT
			Heat				
+ st.	+ Cloudiness	+ st. Flocculent; separates on standing	+ v. sl. Clouding	‡ (?) v. sl. Cloudiness	None or very doubtful	None or very doubtful	+ v. sl. Clouding
			Addition of NaCl after heating				
+ st. Flocculent	+ Fine flocculent precipitate; separates	+ st. Large flocculent precipitate; separates	+ Cloudiness more marked; no floccules separate	+ Cloudiness not marked; no floccules separate	+ st. Marked cloudiness; no particles separate	+ st. Marked cloudiness; no floccules separate	+ st.

* The signs and abbreviations in the table have meanings as follows: (+) precipitate formed; st = strong; v. sl. = very slight; (?) = doubtful.

careful autopsies and microscopic study of the lungs were made on all animals after they died or were killed. If the effects were not immediately fatal, the animals were killed by a blow at the end of about half an hour.

The necropsy was conducted immediately after death and blocks of the lungs, usually both lower lobes, cut not more than 4 mm. thick. These were placed in Zenker fluid for 24 hours, washed in freely running water for twenty-four hours and placed in 70 per cent alcohol. Then they were run through graded alcohols, imbedded in paraffin and stained with hematoxylin and eosin.

The volume of solution injected ranged from 0.5 to 3 cc., and the dosage of the individual agents was varied. In some experiments this was somewhat higher per gram of animal than the therapeutic dosage of the agents. However, the dosage of the thromboplastic agents is very elastic and is frequently advised ad libitum owing to the impression that they are non-toxic. If no effects are demonstrable with maximal doses, none would be expected with small, and the statements made concerning the harmlessness of these agents would be more justified. Therefore, we believe that the use of large doses in these experiments is correct in principle. It should be remembered also that repeated medication with these agents is the rule rather than the exception.

The following summary of doses expressed in milligrams per gram body weight and calculated on the basis of a 60 kilo individual indicates that the dosage recommended or used by others is not seriously different from the higher doses used by us:

Coagulen: 0.083 mgm. per gram intravenously advised in the manufacturer's literature for hemophilia. 1 per cent and 3 per cent strengths are recommended.

Kephalin: 0.125 mgm. per gram intravenously, and 0.05 gram subcutaneously produced a rise of temperature (Lucas and Hurwitz) (3); 0.1 mgm. per gram in dogs intravenously (Howell) (4).

Thromboplastin: 2 cc. of 10 per cent solution in rabbits (Hess) (5); not recommended for use intravenously in human individuals but is used subcutaneously 10 to 20 cc. every twenty-four hours = 0.00033 cc. per gram (maximal).

TABLE 2

Anaphylactoid phenomena from thromboplastic agents intravenously and subcutaneously

NUMBER OF EXPERIMENT	WEIGHT OF GUINEA PIG	VOLUME OF SOLUTION INJECTED	DOSAGE PER GRAM OF ANIMAL (MGM. OR CC.)	SYMPTOMS	FATE OF GUINEA PIG	KILLED END OF	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Thromboplastin (Squibb, fresh)								
	grams	*cc.*				*minutes*		
18	200	3.0	0.015 cc.	Convulsions; relaxation of sphincters; cyanosis; depression	Fatal in 7 min.		Lungs collapsed, cardiac dilatation, general abdominal congestion, pneumonia	Moderate distension, marked congestion, and marked thrombosis
66	360	1.0	0.0028 cc.	Dyspnea; increased respiration; jerky spasms of neck	Recovery	45	Slight distension of lungs; marked pulmonary hemorrhages; marked cardiac dilatation; slight abdominal congestion	Slight distension, marked congestion, slight thrombosis
72	390	0.5 (subcutaneously)	0.0013 cc.	Restlessness; slight dyspnea; increased respiration	Recovery	32	Lungs distended and congested; heart dilated, slight abdominal congestion	Marked distension, marked congestion, marked hemorrhage, conglutination thrombi
73	430	0.5 (subcutaneously)	0.0011 cc.	Deep respiration; no effort	Recovery	37	Marked distension of lungs and marked hemorrhages; cardiac dilatation; slight abdominal congestion	Moderate distension, marked congestion, hemorrhage, conglutination thrombi

Thromboplastin (Armour, fresh)								
64	350	1.0	0.003 cc.	Some dyspnea; increased respiration; ruffling of hair	Recovery	50	Marked distention of and hemorrhage in lungs; slight cardiac dilatation; moderate abdominal congestion	Marked congestion
78	215	1.5	0.007 cc.	Dyspnea marked; increased respiratory rate	Recovery	35	Marked distension of lungs; pulmonary hemorrhages; slight cardiac dilatation, abdominal congestion	Moderate distension, marked congestion, marked hemorrhage
Coagulen (dry, fresh 3 per cent)								
33	275	3.0	0.32 mgm.	Dyspnea; increased respiration	Recovery	35	Marked inflation of lungs, pulmonary hemorrhages, heart slightly dilated, no abdominal congestion	Moderate congestion
41	220	3.0 (ampoule solution)	0.45 mgm.	Dyspnea; marked increase of respiration; restlessness	Recovery	30	Marked inflation of lungs, pulmonary hemorrhages, cardiac dilatation, no abdominal congestion	Marked distension, marked congestion, moderate hemorrhage, thrombosis

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	WEIGHT OF GUINEA PIG	VOLUME OF SOLUTION INJECTED	DOSAGE PER GRAM OF ANIMAL (MGM. OR CC.)	SYMPTOMS	FATE OF GUINEA PIG	KILLED END OF	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Coagulen (dry, fresh 3 per cent)—*Continued*								
	grams	*cc.*				*minutes*		
65	420	3.0 (ampoule)	0.21 mgm.	Dyspnea; respiration slowed; restlessness; spasms	Recovery	46	Slight distension of lungs; marked pulmonary hemorrhages; distinct abdominal congestion; heart markedly dilated	Moderate distension, marked congestion, marked thrombosis
67	400	0.5 (dry-fresh)	0.038 mgm.	Marked dyspnea; increased respiration; restlessness	Recovery	40	Lungs moderately distended and congested; no hemorrhages; marked cardiac dilatation; slight abdominal congestion	Slight distension, moderate congestion, fine conglutination thrombi
69	370	0.5 (ampoule)	0.041 mgm.	Marked dyspnea; increased respiration	Recovery	36	Moderate distension of lungs; pulmonary congestion and hemorrhages; cardiac dilatation; moderate abdominal congestion	Marked congestion

71	430	1.0 (ampoule)	0.07 mgm.	Moderate dyspnea; increased respiration	Recovery	33	Lungs moderately distended and markedly congested; pulmonary hemorrhages; moderate cardiac dilatation; abdominal congestion	Marked congestion
75	320	3.0 (subcutaneously)	0.28 mgm.	Dyspnea; increased respiration; restlessness	Recovery	68	Slight distension of lungs; pulmonary congestion and hemorrhages; marked cardiac dilatation; slight abdominal congestion	Moderate distension, marked congestion, marked hemorrhage
79	350	3.0 (subcutaneously) ampoule	0.26 mgm.	Dyspnea; increased respiration	Recovery	68	Lungs distended; marked pulmonary hemorrhage and congestion; cardiac dilatation; slight abdominal congestion	Marked congestion

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	WEIGHT OF GUINEA PIG	VOLUME OF SOLUTION INJECTED	DOSAGE PER GRAM OF ANIMAL (MGM.) OR CC.	SYMPTOMS	FATE OF GUINEA PIG	KILLED END OF	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Hemostatic serum (fresh; Parke, Davis Company)								
	grams	*cc.*				*minutes*		
26	185	3.0	0.016 cc.	Depression; marked dyspnea; increased respiration; convulsions; shivering	Recovery	38	Lungs collapsed and hemorrhagic; cardiac dilatation, pneumonia, general abdominal congestion	Moderate distension, moderate congestion, marked hemorrhage, thrombosis and edema
68	340	2.0	0.006 cc.	Marked dyspnea; increased respiration; restlessness	Recovery	37	Lungs about collapsed; marked pulmonary congestion; slight cardiac dilatation; abdominal congestion	Slight distension; marked congestion; conglutination thrombi
77	360	1.0 (subcutaneously)	0.003 cc.	Moderate dyspnea	Recovery	84	Marked distension of lungs; pulmonary hemorrhages and congestion; marked cardiac dilatation; abdominal congestion	Marked congestion; slight distension

Kephalin (fresh sheep; 0.1 per cent, 1 per cent and 0.5 per cent)								
8	372	2 cc. of 0.5 per cent	0.027 mgm.	Depression marked	Recovery	60	Lungs collapsed, otherwise normal	Moderate distension, slight congestion, slight hemorrhage
63	220	3 cc. of 0.1 per cent	0.014 mgm.	None	Recovery	34	Lungs somewhat distended and congested, pulmonary hemorrhages, slight cardiac dilatation, moderate abdominal congestion	Marked distension, marked congestion, slight hemorrhage
12	207	3 cc. of 0.1 per cent	0.014 mgm.	None	Recovery	42	Lungs collapsed, slight cardiac dilatation	Slight congestion, slight hemorrhage
76	340	3.3 cc. of 1 per cent	0.1 mgm.	None	Recovery	70	Moderate cardiac dilatation and pulmonary congestion, lungs collapsed	Moderate distension, marked congestion, marked hemorrhage
Trikresol (0.3 per cent in normal saline)								
54	290	3.0	0.031 mgm.	Dyspnea, convulsions, tremors, shivering	Recovery	35	Marked inflation of lungs; hemorrhages, cardiac dilatation, no abdominal congestion	Marked distension, marked congestion, slight hemorrhage

TABLE 2—*Concluded*

NUMBER OF EXPERIMENT	WEIGHT OF GUINEA PIG	VOLUME OF SOLUTION INJECTED	DOSAGE PER GRAM OF ANIMAL (MGM.) OR CC.	SYMPTOMS	FATE OF GUINEA PIG	KILLED END OF	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Trikresol 0.3 per cent in normal saline—*Continued*								
	grams	*cc.*				*minutes*		
59	230	3.0	0.036 mgm.	Tremors; no dyspnea	Recovery	30	Lungs collapsed and congested, no cardiac dilatation, slight abdominal congestion	Marked congestion
74	320	3.0	0.026 mgm.	Tremors; mild convulsions; increased respiration	Recovery	38	Lungs collapsed; marked pulmonary congestion with some hemorrhages; slight abdominal congestion	Moderate distension, marked congestion, marked hemorrhage
Saline controls (0.9 per cent NaCl)								
1	200	2	0.01 cc. (0.09 mgm. NaCl)	None	Recovery	20	Lungs collapsed; otherwise normal.	
7	350	10	0.03 cc. (0.26 mgm. NaCl)	None	Recovery	86	Lungs collapsed; otherwise normal	Slight congestion or normal
46	235	3	0.012 cc. (0.11 mgm. NaCl)	None	Recovery	48	Lungs collapsed; otherwise normal	Marked congestion
52	220	3 (0.85 per cent NaCl)	0.014 cc. (0.12 mgm. NaCl)	None	Recovery	41	Very slight lung inflation and cardiac dilatation	Marked congestion; thrombosis in few large veins

Hemostatic serum: 1 to 2 cc. repeated every four to six hours subcutaneously or intravenously (manufacturer). The maximal dose in twenty-four hours would be 12 cc. or about 0.0002 cc. per gram (0.2 cc. per kilogram).

Kephalin, thromboplastin and coagulen are recommended intramuscularly by Bastedo (6) in dangerous hematemesis.

The results that were obtained with both small and large doses are summarized in table 2.

THROMBOPLASTINS AND HEMOSTATIC SERUM

The following agents which were injected intravenously and subcutaneously into guinea-pigs were found to produce marked anaphylactoid symptoms, the claims of the manufacturers regarding the harmlessness of these agents to the contrary notwithstanding: Thromboplastin (Squibb; fresh), thromboplastin (Armour; fresh) and hemostatic serum. It is probable that the symptoms in part at least were due to the preservative, since all animals injected with equal quantities, that is, 3 cc. of 0.3 per cent trikresol in normal saline showed similar symptoms, though not as marked, and the changes at autopsy were also similar in some of the animals. However, trikresol was not fatal, while thromboplastin was. Presumably, therefore, the proteins are responsible. This is confirmed by the fact that the animals injected with kephalin and normal saline were practically unharmed. The data in table 1 indicate that the thromboplastins and hemostatic serum are rich in proteins, while the kephalins are not.

The changes at autopsy were also more severe than those observed after the injection of saline and trikresol. That is, the thromboplastins and hemostatic serum caused more distention and congestion of the lungs; also hemorrhages and greater cardiac dilatation. There was also more abdominal congestion. The majority of the animals injected with saline showed practically no symptoms and no changes at autopsy, these being somewhat more pronounced after trikresol, but not nearly as marked as after the thromboplastic agents.

Microscopically, the difference between the controls (saline and trikresol) and the thromboplastic agents was even more marked. That is, thromboses in the lungs were quite common with the thromboplastins and hemostatic serum, and entirely absent in the lungs of animals injected with trikresol and only 1 out of 4 animals injected with saline showed few thrombi. The presence of thrombi together with the marked congestion, both abdominal and pulmonary, hemorrhages in the lungs and cardiac dilatation, indicate a serious injury to the circulation.

COAGULEN

Coagulen may be described separately, since it contains no trikresol and is practically protein-free. However, the changes produced after the intravenous and subcutaneous injections of large and small doses of both the fresh dry preparation and the sterile solution in ampules resemble closely those produced by the agents rich in protein (hemostatic serum and the thromboplastins). The respiratory symptoms were more pronounced than with the thromboplastins and hemostatic serum. Dyspnea and lung inflation were more pronounced and there was a greater respiratory rate. The circulatory changes as indicated by hemorrhages, congestion, thrombi and cardiac dilatation were about the same as those with the protein rich agents. The principal difference was the absence of fatalities even with the largest doses used. Nevertheless, the conclusion that coagulen is distinctly injurious, producing definite anaphylactoid symptoms, when injected intravenously, and subcutaneously is justified.

Accordingly, precautions should be exercised in the intravenous and possibly also subcutaneous injection of all these agents, namely, the thromboplastins, hemostatic serum and coagulen. This is contrary to the claims of harmlessness made by the manufacturers.

KEPHALIN

The intravenous injection of kephalin in weak (0.1 per cent) and high concentrations (0.5 per cent), using ordinary doses, produced no demonstrable symptoms in guinea-pigs. The blood

of these animals appeared to clot in about the same way as their fellows injected with saline and other agents. Only one out of the four animals injected showed postmortem evidences of injury to the respiratory and circulatory systems. Microscopically, the pathological changes were also moderate. Kephalin, therefore, is a relatively harmless substance as compared with the other thromboplastic agents studied. Apparently no serious contraindications to its use intravenously exists. When high concentrations are used, the possibility of fat embolism should be born in mind. Neither emboli nor thrombi, clumping of cells or intravascular clotting were observed in the experiments performed.

In this connection it is interesting to note that Bulger (7) regards the change in blood coagulability in anaphylactic shock as due to changes in thromboplastin (kephalin, etc., presumably). If this is true, and since blood in anaphylactic shock has been repeatedly observed to possess a prolonged coagulation time, the injection of thromboplastic agents (kephalin, etc.) theoretically should mitigate the difficulty, if not shock itself. However, our results indicate that certain of these agents produced the signs and symptoms of shock and others did not, and the blood of such animals behaved no differently from that of the controls and other animals used with a series of different agents. It is doubted, therefore, if thromboplastin per se (or thromboplastic agents) plays any causal rôle in the diminished coagulability of blood of anaphylactic shock.

CONCLUSIONS

1. The thromboplastic agents rich in protein (thromboplastin (Squibb and Armour) and hemostatic serum) are distinctly harmful when injected intravenously and subcutaneously in guinea-pigs, producing anaphylactoid symptoms and injury to the circulation; and death with large doses of thromboplastin intravenously.

2. Coagulen, which contains only traces of native protein, produced more pronounced anaphylactoid symptoms and also injured the circulation.

3. The injurious effect of the thromboplastins *and hemostatic serum may be accounted for in part by trikresol (the preservative) and to a greater extent by the protein fraction. This does not appear to be true of coagulen which contains neither trikresol nor native protein.

4. Caution is therefore necessary when employing these agents intravenously and subcutaneously.

5. Kephalin appears to be relatively harmless as compared with the other thromboplastic agents studied.

REFERENCES

(1) Hanzlik and Weidenthal: J. Pharm. Exp. Therap., 1919, 14 :81; 1919, 14 :115.
(2) Lee and Minot: Clev. Med. J., 1917, xvi, 65.
(3) Hurwitz and Lucas: Arch. Int. Med., 1916, xvii, 543.
(4) Howell: Harvcy Lectures, 1916–17, series 12, pp. 273–324.
(5) Hess: J. Am. Med. Assoc., 1915, lxiv, 1395.
(6) Bastedo: Am. J. Med. Sci., 1919, clvii, 99.
(7) Bulger: J. Inf. Dis., 1918, xxiii, 522.

*The Thromboplastin (Squibb) was the ordinary variety, and not that marketed as "Thromboplastin Hypodermic" (Squibb). Since this report was written, we have been informed that the latter is heated. We, therefore, can not assert whether the "hypodermic" produces the same effects as the ordinary thromboplastin.

OBSERVATIONS ON PARADICHLORBENZENE AND PARADIBROMBENZENE

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INTRODUCTION

The toxicity of paradichlorbenzene for earthworms (1) suggests that it may be of value as an anthelmintic and indicates further pharmacologic investigation. The Dow Chemical Company, to whom I am indebted for most of the material, suggested the inclusion of paradibrombenzene which has similar chemical properties, but a somewhat different and less disagreeable odor. The investigation is not completed; but the following data may be placed on record.

PHARMACOLOGIC LITERATURE

We have been unable to find any publications dealing with the pharmacology of these compounds. A popular review of the chemistry and possible uses of paradichlorbenzene is given by Konantz (2). Chemical Abstracts (3) mentions a paper by Duckett, 1915 on its use as fumigant insecticide. M. F. Boyd (4) mentions several papers on its use against pediculi, under the name of global. He also quotes Hase that it blisters skin.

PHYSICAL PROPERTIES OF PARADICHLORBENZENE

This occurs as colorless crystals, melting at 53°C. and boiling at 172°C. (2).

It is nearly insoluble in water, but soluble in benzene, alcohol, ether, or carbon tetrachlorid (2).

When triturated with 10 parts of olive oil or liquid petrolatum, it dissolves almost entirely. The solution is complete by heating, and it does not separate on cooling.

PHYSICAL PROPERTIES OF PARADIBROMBENZENE

This also occurs as colorless crystals. Van Nostrand states its boiling point as 89.3°C. It is soluble to 14 per cent in alcohol. In general, its solubility is presumably similar to that of the chlorin compound.

ODOR AND TASTE

Paradichlorbenzene has a penetrating and persisting odor, reminding of naphthalen; and a somewhat sharp and persistent taste.

When diluted with liquid petrolatum or olive oil to 1:10,000, the odor and flavor are still quite distinct, and resemble bitter almond. It might perhaps be used as a substitute for nitrobenzol in soaps, shoe-blacking, etc., especially as it is much less toxic.

With 1:1000 in oil, the taste is quite disagreeably strong.

Paradibrombenzol has a much weaker odor, and the taste is not repulsive.

CHEMICAL REACTIONS

These compounds are very inert chemically. The chlorine compound does not give color reactions with the following reagents:

Ferris chlorid, with or without chloroform.

Concentrated nitric acid.

Concentrated sulphuric acid, alone, or with ferric chlorid or with formaldehyde.

TOXICITY FOR EARTHWORMS

The concentration of the *chlor* compound that is fatal to earthworms in twenty-four hours was reported as between 0.001 to 0.009 per cent (1). This places it in the class of active an-

thelmintics, i.e., parallel with aspidium oleoresin, chenopodium oil, carvacrol, pelletierin, santonin and thymol.

The toxic concentration of the *brom* compound lies about 0.01 per cent, that is it is almost as effective.

Three worms were placed in 0.01 gram in 100 cc. of water unfiltered. After twenty-four hours, two were dead and one much depressed.

Three worms placed in 0.001 gram in 100 cc. were normal for three days, when the observations were stopped.

ANTHELMINTIC EFFICIENCY IN DOGS

It was attempted to test the anthelmintic efficiency on dogs. The results were very poor; but this is really inconclusive, since anthelmintics that are very effective in man are inefficient against most parasites in dogs, as may be seen from table 1.

TABLE 1

Anthelmintic efficiency on dogs

	DOSE PER ANIMAL (AVERAGE ABOUT 10 KGM.	TAPE-WORM (DIPYLIDIUM CANINUM)	ASCARIS	WHIP-WORMS	HOOK-WORMS
	grams				
Thymol	1		1 = 0		
Chenopodium oil	1	1 = 0	1 = 0		
Betanaphthol	1	1 = 0	1 = 0	1 = 0	
Carvacrol	1–1.2		1 = +	4 = 0	
			1 = 0		
Paradichlorbenzol	1	1 = 0	1 = ±		
			2 = 0		
	15		1 = 0	1 = 0	1 = 0
Paradibrombenzol	1	2 = 0		2 = 0	

The numbers refer to the number of infested animal tried with each drug. 0 = inefficient. + = completely efficient. ± = partially efficient.

Technic of administration. Practically all of the experiments were made according to the following routine, covering four days.

First day. In the afternoon, the animals were taken from stock. They received by stomach tube the first dose of cathartic, namely 50 cc. of a 1 per cent suspension of phenolphthalein in liquid petrolatum.

(This dose was modified for extra large or small animals.) They were then placed in metabolism cages, for the collection of feces. They were given the usual ration of food and water.

Second day. On the afternoon, a second dose of the same cathartic was given.

Third day. In the afternoon the food was withdrawn. The feces up to this time were collected and examined for parasites and ova. The animals were then given, by stomach tube, the anthelmintic drug, dissolved or suspended in 25 cc. of liquid petrolatum, and mixed with 25 cc. of castor oil (the two oils separate on standing).

Fourth day. In the morning, the feces were collected and examined for parasites. The dogs were then used in class work and killed about noon. The postmortem was made within two hours. The intestines were opened for their entire length and examined for parasites.

Results with paradichlorbenzol. This was given to three infested dogs with doses of 1 gram; and to one dog with 15 grams. A *partial success* was secured against *ascaris* in one case; in three other dogs, including the 15 gram dose, it was *ineffective* against ascaris. It was also *ineffective* (in one animal respectively) against *tapeworm* (dipylidium caninum) (1 gram), *hookworm* (15 grams) and *whipworms* (15 grams).

Dog 42.

Dose: 1 gram.

Administration: Routine, but killed after eight days.

Parasites: Stools, on day after administration, contained 2 ascaris. Postmortem, after eight days, revealed 4 ascaris remaining in intestines.

Efficiency: Partial against ascaris.

Dog 4

Dose: 15 grams.

Administration: The drug was suspended in 100 cc. of 25 per cent magnesium sulphate given by stomach tube. The animal was killed after two days.

Parasites: Postmortem, ascaris, hookworm, and whipworms.

Efficiency: None against ascaris, hookworm and whipworm.

Dog B

Dose: 1 gram.

Weight: 8.5 kgm.

Administration: Routine.

Parasites: Stools (free catharsis) contained ascaris ova, but not worms.

Efficiency: None against ascaris.

Dog 16

Dose: 1 gram.

Weight: 9.7 kgm.

Administration: Routine.

Parasites: Postmortem, 5 tapeworms and 1 ascaris.

Efficiency: None against tapeworms and ascaris.

Results with paradibrombenzol. This was used (1 gram) in 2 dogs. In both it was inefficient against *tapeworms and whipworms.*

Dog 17

Dose: 1 gram.

Administration: Routine.

Parasites: Postmortem, 32 tapeworm heads and 2 whipworms.

Efficiency: None against tapeworms and whipworms.

Dog 18

Dose: 1 gram.

Administration: Routine.

Parasites: Postmortem, 6 tapeworms and 2 whipworms.

Efficiency: None against tapeworms and whipworms.

SYMPTOMS AND LESIONS IN DOGS

Table 2 shows the dosage and the period before the dogs were killed. None of the animals showed any of the toxic symptoms during their life. Dog 2 was found dead after a week, but presumably had been killed by the other dogs in the kennel.

The postmortem examination also failed to reveal abnormalities attributable to the drugs. Dogs 2 and 5 were entirely normal; dog 16 had slight congestion of the liver and small and large intestines; with normal stomach and kidneys. In dog 4, the intestines were somewhat hemorrhagic, doubtless due to the hookworms.

TABLE 2

Dosage of dichlorbenzene and dibrombenzene. The drugs were given by stomach tube

EXPERIMENT NUMBER	DOSE, PER KILOGRAM	WEIGHT OF DOG	TOTAL DOSE	DAYS BEFORE KILLING	VEHICLE
Paradichlorbenzene					
	grams	*kgm.*	*grams*		
4	1.5*	10.0*	15	1	100 cc. 25 per cent magnesium sulphate
2	0.77	6.5	5	1	25 cc. castor oil
5	0.5*	10.0*	3	1	50 cc. castor oil and 25 cc. liquid petrolatum
B	0.12	8.5	1	3	25 cc. castor oil and 25 cc. liquid petrolatum
16	0.10	9.7	1	1	25 cc. castor oil and 25 cc. liquid petrolatum
12	0.10*	10.0*	1	1	25 cc. castor oil and 25 cc. liquid petrolatum
Paradibrombenzene					
17	0.10	10.0*	1	1	25 cc. castor oil and 25 cc. liquid petrolatum
18	0.06	18.0	1	1	50 cc. castor oil and 25 cc. liquid petrolatum.

* Approximate.

ABSORPTION OF BROMIN FROM PARADIBROMBENZENE

The poor solubility and reactivity of these compounds made it doubtful whether they would be absorbed from the alimentary tract, and therefore, whether they would be likely to produce systemic effect. The appearance of bromin compounds in the urine would probably be the most delicate index of absorption. Only one experiment has been made so far. This showed an unmistakable but very slight, absorption equivalent to about 3 mgm. of paradibrombenzene, or $\frac{1}{3}$ per cent of the quantity administered.

Dog 18, weighing 18 kgm., received 1 gram of paradibrombenzene in 100 cc. of oil by stomach tube, and was killed after eighteen hours. The bladder contained 50 cc. of urine, which was withdrawn, and tested by Mr. N. C. Wetzel.

The test consisted in the addition of dry chlorinated lime and concentrated hydrochloric acid, and shaking out with carbon disulphid.

The disulphid was not colored when the test was applied directly to 10 cc. of the urine. The remaining 40 cc. of urine were evaporated, incinerated with sodium carbonate and dissolved in 100 cc. water, and tested. The disulphid became slightly yellow. The color was matched by adding to a control-test a 1 per cent solution of KBr. This required 5 drops, i.e., about 3 mgm. of KBr or 2 mgm. of Br; or 2.5 mgm. of Br for the entire urine. Since the 1 gram of paradibrombenzene administered contained 678 mgm. of Br, the absorption amounts to about 0.37 per cent.

ATTEMPT TO RECOVER PARADICHLORBENZENE BY EVAPORATION OF SOLVENT

In the hope of evolving a method of separating paradichlorbenzene from excreta by solvents, it was first attempted whether it could be recovered from the solutions in carbon tetrachlorid or ether by evaporating the solvent. The results are not promising, the chlorine compound being apparently too volatile.

The experiments were made as follows: 0.1 gram of the drug was dissolved in 100 cc. of solvent, in a tared dish. It was then evaporated on a water-bath to about 10 cc., then permitted to dry spontaneously at air temperature and weighed. After standing another day uncovered in the air, it was weighed again. The following results were obtained by Miss J. R. Collacott:

	WITH CARBON TETRACHLORID	WITH ETHER
Weight of residue after evaporation	75 mgm. = 75 per cent	50 mgm. = 50 per cent
Weight of residue after one day	20 mgm. = 20 per cent	Nil

CONCLUSIONS

Paradibrombenzene has a less disagreeable odor and taste than paradichlorbenzene, and would therefor be more suitable for internal administration.

Both compounds have a high toxicity for earthworms, and may therefor be effective anthelmintics. Experiments were tried

on dogs but this method is inconclusive. The substances deserve clinical trial when their toxicity has been more fully determined. So far, no toxic effects have been observed from oral administration, even of very large doses. Their absorption is probably very slight. In one experiment with paradibrombenzene, the absorption, judged by the bromin excretion, was only 3 mgm. after the administration of 1 gram.

REFERENCES

(1) Sollmann: Jour. Pharm. Expt. Therap., xii, 129.
(2) Konantz: Am. Jour. Pharm., 1918, ix, 340.
(3) Duckett: Chemical Abstracts, x, 3114.
(4) Boyd, M. F.: Internat. Assoc. Med. Museums Bull. VII, 1918

EXPERIMENTS WITH CARVACROL

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INTRODUCTION

Carvacrol is an isomer of thymol, which can be produced at a low cost from spruce-turpentine, according to the procedure patented by R. H. McKee (1). It is said to be actively germicidal, and has been used as a counterirritant anesthetic against toothache. From its composition, it seems probable that it might be used as an anthelmintic, especially against hookworm, in the place of the much more expensive thymol.

Dr. McKee suggested that anthelmintic efficiency be tested on earthworms, and supplied the material. The results seemed sufficiently promising to justify further preliminary investigation of efficiency and toxicity. The results indicate that the substance is probably an available anthelmintic although the method of investigation did not give conclusive results. Its acute toxicity is not very high; but it is an active intestinal, hepatic and renal irritant and must be used with caution.

The work was not carried further, since an investigation of its toxicity had been planned at another laboratory.

TABLE 1.

Miscibility with aqueous media

AGENT	CARVACROL (0.1 cc. IN 10 cc.)		TURPENTINE OIL (0.1 cc. IN 10 cc.)	
	Results at once	On standing	Results at once	On standing
Water	Insoluble, but remains suspended easily	Separates very slowly		
Sodium chlorid, 0.9 per cent	Insoluble, but remains suspended easily	Separates very slowly	Separates at once	
Sodium bicarbonate, 0.5 per cent in 0.9 per cent NaCl	Insoluble, but remains suspended easily	Turns pink, very slowly		
Sodium bicarbonate, 1 per cent in 0.9 per cent NaCl	Insoluble, but remains suspended easily	Turns pink, very slowly		
Sodium carbonate, 1 per cent	Insoluble, but remains suspended easily	Turns pink at once, but no deeper than with bicarbonate. Solubility no greater than in water		
Serum, 1 per cent in 0.9 per cent NaCl	White precipitate		No precipitate. Suspends well	Separates slowly

Defibrinated blood (Beef)	Darkens, then lakes promptly	Precipitates slowly	Remains suspended	Does not lake in several hours. No precipitate
Milk (skimmed)	Suspends easily	Apparently remains suspended (or precipitates casein ?)	Remains suspended	
Acacia (25 per cent)	Suspends easily	Precipitates slowly	Remains suspended	No precipitate
Starch (U.S.P.T.S.)	Suspends easily			
Alcohol 25 per cent in N.S. 0.9 per cent NaCl	About like N.S.		Separates at once	

EXPERIMENTAL WORK ON CARVACROL

A. Miscibility with aqueous media

This was determined with a view to its possible use in the administration. The experiments were made by shaking 0.1 cc. of carvacrol with 10 cc. of the menstruum. Data with turpentine oil in the same proportions are given for comparison.

Carvacrol is almost insoluble in water, but since it has nearly the same specific gravity (0.978), it suspends easily in water, normal saline, or bicarbonate. Alkaline suspensions turn pink, indicating chemical change. The miscibility is not materially improved by the addition of 25 per cent of alcohol.

Proteins and acacia are not necessary for suspension, and are rather objectionable, since they tend to precipitate slowly. Blood is laked.

Turpentine oil does not mix with water or 25 per cent alcohol; but requires the addition of proteins or acacia. It does not precipitate these, nor lake blood.

The details are shown in table 1.

B. Precipitation of proteins

The precipitant effect of carvacrol on proteins, is of pharmacological interest. It indicates, for instance, that it would be irritant. Comparative experiments were therefore made with some substances related in constitution and action. It was found that carvacrol is more actively precipitant, and therefore presumably more irritant than the other substances tried, the order being:

Most:
- Carvacrol
- Chenopodium oil
- Phenol
- Chloroform
- Thymol

Least:

The tests were made by mixing equal volumes of 1 per cent watery solutions or suspensions of the agents with the proteins and shaking. The precipitates formed as shreds.

Acacia (25 per cent) and starch (U. S. P. test solution) did not precipitate, but formed more permanent suspensions than the aqueous vehicle. The results with the proteins are shown in detail in table 2.

TABLE 2

Precipitation of proteins

	SOLUBILITY	1 PER CENT AQUEOUS SOLUTION OR SUSPENSION	EGG ALBUMEN, 1:10	FRESH DOG SERUM, 1:10	DRIED BLOOD SERUM, 1:100	PEPTONE, 5 PER CENT
1		Control	–	–	–	–
2	–	Thymol (on standing)	–	T–	–	T–
3	±	Chloroform	+	–	±	–
4	+	Phenol	±	±	+	±
5	–	Oil chenopodium (slowly on shaking)	++	+	++	+
6	–	Carvacrol	++	±	++	++

+ = precipitate; ± = doubtful precipitate; T = turbidity; – = no precipitate.

C. Hemolysis

Carvacrol is a very active laking agent. When defibrinated blood is added to a 1 per cent suspension of carvacrol in normal saline in a test tube, or on a slide under the microscope, laking occurs immediately. Turpentine or olive oil do not produce this effect.

Experiments were made by Mr. G. E. Richardson on some other phenols and volatile oils, by adding 2 drops of defibrinated blood to 5 cc. of the solutions or suspensions, made up with 0.9 per cent sodium chlorid. The results show that none of these approach carvacrol in the destructive effects on blood-corpuscles.

Thymol. This seems to hemolyze if it is well mixed with the solution.

Phenol. This lakes in concentrations of 0.3 to 1.25 per cent and weaker; stronger solutions do not lake, and prevent laking by water, presumably by precipitating the cell envelope.

Guaiacol, turpentine oil and chenopodium oil. These do not produce laking in 2 per cent.

Lavender oil. Two per cent produces apparently a slight hemolysis.

Details of thymol and phenol

Thymol. A crystal shaken with 5 cc. of 9 per cent NaCl: No change in an hour. When the crystal was first dissolved in a little alcohol and then added to the saline, laking occurred within ten minutes.

Phenol. Test tube, solutions in 0.9 per cent NaCl:

0.3 per cent, very slight hemolysis in an hour.

0.6 per cent, nearly hemolyzed in an hour, completely in one and one-quarter hours.

1.25 per cent, hemolysis started at once and appears complete in one-half hour.

2.5 per cent, immediate salmon colored turbidity same in an hour. Cells smaller and somewhat paler. Not laked by water.

5.0 per cent, immediate brown precipitate; microscopically, cells are clumped, but of normal color. Not laked by water.

Turpentine oil. 2 per cent, test tube: No hemolysis in an hour.

Chenopodium oil. 2 per cent, no hemolysis.

Lavender oil. Slight hemolysis.

D. Toxicity for earthworms

The fatal concentration lies at 0.001: 100. Its efficiency is therefore about the same as that of thymol, oil of chenopodium, oleoresin of aspidium, pelletierin tannate, etc. (Sollmann (2)). It is therefore presumably a very active anthelmintic.

Method. 0.013 gram of carvacrol was rubbed with talcum and 130 cc. of water = 0.01 per cent. This is used without filtering, as such and as further dilutions. 100 cc. of each solution are placed in conical glasses and 3 earthworms added.

Results. All show immediate agitation.

0.01: 100 = practically dead in forty minutes.

0.001: 100 = much depressed in two hours; just dead in twenty hours.

0.0001: 100 = normal in two hours and in twenty hours.

E. Anthelmintic efficiency for dogs

The attempt was made to estimate anthelmintic efficiency by administration to dogs. From 1 to 1.2 grams together with cathartics were given by stomach to fasting infested animals. The administration was successful against ascaris in one animal; unsuccessful in another. It was unsuccessful against whipworms in four dogs. These results were fully as successful as those with any of the standard anthelmintics that were tried. In fact, the efficiency of anthelmintics in dogs is so poor and so uncertain that further experiments were abandoned. Apparently, the anthelmintic efficiency of carvacrol can only be determined by experience on human subjects.

TABLE 3

Gastric administration of carvacrol

DOG NUMBER	DOSE*	WEIGHT OF DOG	DOSE PER KILOGRAM	TIME BETWEEN ADMINISTRATION AND KILLING OF ANIMAL
	cc.	*kgm.*	*cc.*	
11	1.2	13.3	0.090	3 days
14	1.0	9.7	0.103	3 days
(A) 4	1.0	9.0	0.111	2 days
1	5.0	9.6	0.526	3 weeks

* Given by stomach tube, in a mixture of 25 cc. of liquid petrolatum and 25 cc. of castor oil.

F. Toxicity for dogs, gastric administration

Carvacrol, when administered by stomach tube in oily solution, does not have a high acute toxicity. The only immediate effect was in a dog that received 15 cc. of carvacrol in 90 cc. of castor oil, and which vomited within five minutes. It is doubtful whether this emesis was caused by the carvacrol, since it was absent in all the other dogs.

These received the doses noted below in table 3. In none of these were any symptoms noted during the life of the animals. Those in which a postmortem examination was made, however, showed marked congestion of the small intestines from the duodenum to the ileum, and of the kidneys and liver. The

stomach and colon appeared normal. From this it is apparent that large doses of carvacrol produce strong irritation of the intestines and other abdominal viscera.

As we understood that the toxicity of carvacrol was under investigation at another laboratory, we did not continue further work in this line.

CONCLUSIONS

Carvacrol, a synthetic isomer of thymol, readily forms suspensions with aqueous fluids. These are rendered more permanent by acacia or starch.

Carvacrol precipitates proteins more actively than do chenopodium oil, phenol, chloroform or thymol. These precipitate in descending order.

Carvacrol is an active laking agent, more powerful than thymol or phenol.

Carvacrol has a high toxicity for earthworms, ranking with thymol, chenopodium oil, aspidium oleoresin, pelletierin tannate, etc. It is therefore presumably an active anthelmintic.

Experiments on dogs demonstrate that carvacrol is effective against ascarids. However, dogs proved unsatisfactory test-animals.

The gastric administration of carvacrol to dogs in doses up to 0.5 per kilogram (corresponding to about 30 cc. per man) produced no toxic symptoms within three weeks. However, much smaller doses (0.1 cc. per kilogram equivalent to 5 cc. per man) apparently produced marked congestion of the small intestines, liver and kidneys. It is probably more irritant and toxic than thymol. This enjoins caution in its use.

The drug seems to deserve clinical trial as an anthelmintic substitute for thymol. The administration, however, must be worked out cautiously. The routine should be the same as for thymol, and the dosage should be started considerably smaller, until the zone of safety has been determined.

REFERENCES

(1) Hixson, A. W., and McKee: J. Ind. Eng. Chem., x, 982, 1918.
(2) Sollmann: J. Pharmacol., xii, 129, 1918.

AN EXPERIMENTAL STUDY OF THE ACTION OF CHLORAMINES

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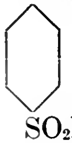
Though a great deal has been written, of late, on the chloramines, first prepared by Kastle, Kaiser and Bradley (1), further studied by Chattaway (2) and made prominent by H. D. Dakin (3) and his associates, we are not acquainted with any systematic studies of the action of these substances upon animals. In view of the probable practical importance of these bodies of high disinfectant value, we undertook to study their effect upon various forms of animal life.

I. SODIUM P-TOLUENE SULPHONCHLORAMINE (CHLORAMINE-T)

This substance, a white crystalline body of faint chlorinous odor and bitter taste, freely soluble in water, its saturated aqueous solution containing 15 per cent of the salt, has the following structural formula:

CH_3



SO_2NaNCl.

It is faintly alkaline in reaction. It does not precipitate or coagulate protein.

Action on protozoa. The protozoan culture used was obtained by placing a little fresh horse manure in water for some days, when it was found swarming with infusoria, chiefly stylonychia and vorticella. Exposing this culture to various concentrations of chloramine-T, we obtained the following results:

Action upon stylonychia: Concentrations up to 1:20,000 stop all motion almost instantly; 1 : 40,000 stops all motion within ten minutes. 1: 50,000 does not stop motion. From this it will be seen, that this substance kills stylonychia in strengths of 1 : 40,000. Vorticella was, however, more resistant. It required 1 : 5000 to stop its motion completely; while 1 : 10,000 and 1 : 20,000 caused the body of the organism to assume a ball form and become immobile, while the stem continued capable of contraction into a spiral.

Effect upon frogs. A frog injected into the anterior lymph sac with 0.4 mgm. of the substance per gram body weight will show evidence of depression, commencing within ten minutes. Turned over, the animal remains upon its back. Next respiration becomes depressed, and finally is abolished. The skin overlying the anterior lymph sac becomes greatly inflamed within half an hour. Usually, within an hour, the reflexes are abolished; and the heart finally stops in diastole. Electric stimulation of the peripheral nerves and muscles still elicits responses. We have, therefore, a descending paralysis of the central nervous system. In addition, there is marked local irritation. It is probably owing to this and the necrosis of the tissues overlying the area of injection, that animals having received smaller doses succumb, even after the lapse of a week, as is shown by the following summary of experiments:

DOSE	RESULTS
mgm. per gram	
0.2	Of five animals two died in one day, the others died in six, nine, and ten days respectively
0.3	Three died in about one and one-half hours, one in four hours, and one after one day
0.4	Three died within one hour, one within eight hours, and one after five days

All these doses were fatal; but, the smaller the dose, the longer the animal survived.

Effect upon mice. White mice, receiving a hypodermic injection of doses of 0.3 mgm. per gram in 1 per cent aqueous solu-

tion, show after a stage of excitation, usually within ten minutes, evident depression. In the course of an hour or two, the animal, placed on its back, turns over with great difficulty or not at all. Respiration becomes slow and labored, and cyanosis quite evident. The animal usually dies within twenty-four hours. Table 1 shows the final results from varying doses.

TABLE 1

DOSE	NUMBER OF EXPERIMENTS	RESULT
mgm. per gram		
0.1	4	3 recovered
0.15	5	2 recovered
0.20	3	2 recovered
0.30	4	1 recovered
0.50	1	Died

The minimum lethal dose (hypodermic) for mice is probably about 0.3 mgm. per gram.

Effect on guinea-pigs. The subcutaneous injection of a 10 per cent solution is evidently painful. After the excitation due to the injection has subsided, the animal receiving a dose of 0.9 gram per kilogram shows evidence of depression. The hind limbs especially, become weak after a time, and the animal remains on its back when turned over. Death occurs in the course of several hours, or else the animal recovers with a patch of induration at the point of injection, which is very liable to undergo necrosis. Necropsy of the animals that have succumbed to the poison, shows nothing remarkable excepting edema and sometimes hemorrhages in the area of injection. The blood shows the spectrum of oxyhemoglobin.

Taylor and Austin (4) abandoned the subcutaneous injection of chloramine-T for the determination of the fatal dose as unsatisfactory. However, they employed only two animals, giving them up to 0.5 gram per kilogram, both of which recovered. We believe that we have used a sufficient number of animals, with sufficient uniformity of results, to feel certain that it is possible to determine the lethal dose by this method.

As will be seen from table 2, the minimal lethal dose, on subcutaneous injection, is 0.9 gram per kilogram, which is not much more than that of phenol which, for this species of animal, seems to be about 0.7 gram per kilogram (table 3). Hence, for the guinea-pig, this substance is not much less toxic than is phenol.

TABLE 2

Toxicity of chloramine-T for guinea-pigs

DOSE (HYPODERMIC)	NUMBER OF EXPERIMENTS	RESULT
gram per kgm.		
0.2	3	All recovered
0.3	3	2 recovered
0.4	3	2 recovered
0.6	3	All recovered
0.7	3	2 recovered
0.8	3	2 recovered
0.9	5	3 died
1.0	4	All died

The minimum lethal dose (hypodermic) for guinea-pigs may be stated to be 0.9 gram per kilogram.

TABLE 3

Toxicity of phenol for guinea-pigs

DOSE (HYPODERMIC)	NUMBER OF EXPERIMENTS	RESULT
gram per kgm.		
0.25	1	Recovered
0.35	1	Recovered
0.40	3	All recovered
0.50	6	2 died, 4 recovered
0.60	5	2 died, 3 recovered
0.70	1	Died
0.75	1	Died

The minimum lethal dose (hypodermic) of phenol for guinea-pigs seems to be 0.7 gram per kilogram.

Whether this is also true for man remains to be seen. The comparison given may be of interest in an attempt to establish a scale of relative toxicities of disinfectants, alongside of the determination of the phenol coefficient.

Effect upon rabbits. The local irritant action of drugs upon mucous membranes may be conveniently studied by means of

the rabbit's conjunctiva. For this purpose, we employed the following technic: The solution was kept in contact with the membrane by pulling the lower eyelid outward and upward by means of forceps and filling with the solution the pouch thus formed. After an exposure of exactly one minute, the solution was permitted to escape. Nothing further was done, excepting to observe the eye from time to time.

In this manner it was found that a 1:1000 solution of chloramine-T is distinctly irritating, while a 1:2000 solution is not. Phenol, used for comparison, showed distinct irritation in solution of 1:300, while 1:500 was negative or but slightly in effect. Chloramine-T, is, therefore, about three times as irritant as is phenol.

The effect of this substance upon the rabbit on intravenous injection were studied in varying dosages by means of slow injection of a 10 per cent solution into the rabbit's ear vein. The results are shown in table 4.

TABLE 4

Intravenous injection of chloramine-T into rabbits

DOSE	NUMBER OF EXPERIMENTS	RESULTS
gram per kgm.		
0.025	1	Died in 2 hours
0.050	1	Recovered
0.075	2	Both died
0.100	1	Died in 1 hour
0.150	1	Died in 10 minutes

The symptoms displayed by animals receiving intravenous injections of chloramine-T, in amounts ranging from 0.075 to 0.150 gram per kilogram in 10 per cent solution, may perhaps best be described by means of the following typical protocol:

Rabbit 2, weighing 2.1 kgm., was given at 10.30 a.m. an intravenous injection of 0.210 gram of chloramine-T in 10 per cent solution. There was no obvious change until 11.30 a.m., when the animal appeared cyanotic and leaned over to one side. At 11.33 a.m. the animal was taken with a violent convulsion, during which bloody foam escaped from its nose and mouth. The animal now became pallid, and at 11.34,

its respiration stopped. At 11.35, the heart beat stopped. Necropsy revealed lungs crepitating with fluid, otherwise nothing marked. The blood showed the oxyhemoglobin spectrum. The cause of death was apparently pulmonary edema. On microscopic examination, a greater desquamation of the alveolar epithelium seemed to be present than in the normal.

With doses of 0.150 gram per kilogram the latent period was but a few minutes, otherwise the phenomena were the same.

It should be noted that such symptoms as cyanosis and pulmonary edema never occur when the poison is given subcutaneously or by mouth.

That the pulmonary edema is due to the chlorine in the molecule is suggested by the fact that, when a rabbit weighing 1900 grams was injected intravenously with 0.190 gram of sodium p-toluene sulphonamine, no symptoms of pulmonary edema developed. The animal showed depression of the central nervous system, from which it however recovered. The only difference between the molecule of this latter substance and that of chloramine-T is an atom of chlorine.

To study the question of excretory irritation, several rabbits weighing about 2000 grams each, were given daily subcutaneous injections of 10 cc. of 10 per cent solution of chloramine-T for about a week. Excepting for swellings developing at the points of injection, there were no obvious effects. The urine remained normal, excepting that it would give a cloud with nitric acid, not due to albumin, however, but to a decomposition product of chloramine, probably p-toluene sulphonamine, for alcohol prevented the precipitation, and heat did not cloud the urine. On microscopic examination of the kidney, liver, heart and lungs, of such animals, nothing abnormal was found. The subcutaneous tissue at the site of injection showed great separation and some swelling of its component fibers, with a moderate degree of round cell infiltration.

Effect on dogs. On oral administration, the phenomena of local irritation are most prominent. Thus, a dog, weighing 8 kgm., was given 85 cc. of 4 per cent solution of chloramine-T dissolved in 0.9 per cent sodium chloride solution. The result

was profuse and repeating emesis, commencing almost immediately, and repeating itself six or eight times within thirty minutes. Several days later, the same dog received 180 cc. of 1:250 chloramine-T. The only phenonema noted were emesis seven hours later; also profuse soft bowel movements on the same day. The difference in concentration was evidently the determining factor in the difference of the results of the irritation in the two experiments.

Intravenous injection of 1 cc. of 10 per cent solution into a 5 kgm. dog, under light ether anesthesia, had practically no effect. Slow intravenous injection of 30 cc. of 10 per cent solution of chloramine-T in normal saline into a 30 kgm. dog, under light ether anesthesia, produced a slowing and weakening of the heart beat; and, within nine minutes, large amounts of serosanguinolent froth issued from nose and mouth. The heart then became extremely slow; and, within twenty minutes, death took place, preceded by a general convulsion. On necropsy, the lungs showed pulmonary edema.

The blood from this dog, as well as that from another dog, weighing 15 kgm., that received over 1 gram of chloramine-T intravenously, showed the oxyhemoglobin spectrum.

Action on blood. Fear has been expressed by various authors that chloramines might be dangerous for purposes of wound irrigation by reason of their tendency to produce methemoglobin. Hence the effect of these bodies upon blood was carefully studied.

Dog's and rabbit's blood were employed in this work. Table 5 shows a comparison of the hemolytic effect of chloramine-T with that of alkali. It will be noted that the hemolysis is considerably slower in case of chloramine-T. This is evidently due to slower dissociation and formation of OH-ions.

The explanation for the change in color noted in these experiments was searched for by means of the spectroscope. Table 6 shows the results from a typical experiment of this kind, in which was employed blood in dilution of 1 :250 that had showed the oxyhemoglobin spectrum well. When this was treated with chloramine-T the oxyhemoglobin bands disappeared after a variable length of time. That the disappearance of the oxyhem-

TABLE 5

Comparative effect of chloramine-T and of alkali upon blood suspension in vitro

DILUTION IN 0.9 PER CENT OF NaCl	HEMOLYSIS COMPLETE IN	REMARKS
Chloramine-T		
1: 200	55 minutes	Before laking color becomes brownish
1: 400	1 hour, 15 minutes	Before laking color becomes brownish
1: 600	1 hour, 30 minutes	Before laking color becomes brownish
1: 800	1 hour, 45 minutes	Before laking color becomes brownish
1: 1000	2 hours	Before laking color becomes brownish
1: 1600	3 hours, 20 minutes	Before laking color becomes brownish
1: 3200	4 hours	Before laking color becomes brownish
1: 6400	5 hours	Before laking color becomes brownish
1: 12800	6 hours	Before laking color becomes brownish
1: 25600	Incomplete in 22 hours	
1: 51200	No hemolysis in 24 hours	
Control	No hemolysis in 24 hours	
Blood suspension with alkali		
N/100 KOH	10 minutes	Spectroscope shows alkaline hematin in 1 hour
N/200 KOH	15 minutes	Spectroscope shows oxyhemoglobin 24 hours later
N/400 KOH	20 minutes	Spectroscope shows oxyhemoglobin 24 hours later
N/800 KOH	40 minutes	Spectroscope shows oxyhemoglobin 24 hours later
N/1600 KOH	Partial in $3\frac{1}{2}$ hours Complete within 20 hours	
N/3200 KOH	No hemolysis in 24 hours	
N/6400 KOH	No hemolysis in 24 hours	
N/12800 KOH	No hemolysis in 24 hours	
Control	No hemolysis in 24 hours	

oglobin bands is due to the formation of alkaline hematin, the spectrum of which is not shown in this dilution, is proved by the fact that, upon adding Stokes' Reagent, which changes alkaline hematin to reduced alkaline hematin, or hemochromogen, the band of this latter substance appeared very distinctly.

The fact, that a certain amount of methemoglobin is also formed, is shown by table 7, in which much more concentrated hemoglobin solution was used than in the previous experiment, as the methemoglobin band is only well shown in comparatively concentrated solution.

TABLE 6

Spectroscopic examination of laked blood (dilution 1: 250) mixed with chloramine-T

DILUTION	10 MINUTES	50 MINUTES	$2\frac{1}{2}$ HOURS	$5\frac{1}{4}$ HOURS
1:30000		x	x	x
1:20000		x	x	x
1:10000		x	x	Slow
1:8320		x	x	Slow
1:6660		x	x	Very slow
1:5000		x	x	Very slow
1:4660		x	x	Almost 0
1:4330		x	Slow	Almost 0
1:4000		x	Slow	Almost 0
1:3660		x	Very slow	Almost 0
1:3330		x	Very slow	0
1:3000		x	Almost 0	0
1:2660		x	Almost 0	0
1:2330		x	0	0
1:2000		Slow	0	0
1:1660		Almost 0	0	0
1:3330	0	0	0	0

x means oxyhemoglobin bands; 0 means the disappearance of these bands. Addition of Stokes' Reagent produces in one to one and a half minutes the spectrum of reduced alkaline hematin (hemochromogen), with bands between 10 and 11 and between $11\frac{1}{2}$ and $12\frac{1}{2}$.

The production of methemoglobin by chloramine-T is, however, slow and slight, when compared with that from chlorine water; which, when added to 5 per cent defibrinated blood in normal salt solution, turns it brown almost immediately and shows the spectrum of methemoglobin. When half strength chlorine water is added, the change of color occurs within a few

minutes. With one-fourth strength chlorine water, the change of color occurs after twenty minutes. On dilution of any one of the chlorine solutions showing methemoglobin, the methemoglobin band disappeared to give place to the oxyhemoglobin bands.

We were unable to find methemoglobin by spectroscope in the blood of the animals acutely poisoned with chloramine-T, no matter how large a dose was given intravenously or how strong a blood solution we examined. Though it is probable that some is formed under these circumstances, we were unable to demonstrate it. It is, on the other hand, evident that methemoglobin formation does not occur to a sufficient extent in vivo

TABLE 7

Spectroscopic examination of laked blood (diluted 1: 10) mixed with chloramine-T

DILUTION	25 MINUTES	40 MINUTES	24 HOURS
1: 20000	x	x	x
1: 10000	x	x	Very faint methemoglobin
1: 5000	x	x	Faint methemoglobin
1: 2000	x	x	Faint methemoglobin
1: 1000	x	Very faint methemoglobin	
1: 500	x	Very faint methemoglobin	
1: 200	Alkaline hematin	Very faint methemoglobin	

x means oxyhemoglobin. All tubes gave after twenty-four hours the spectrum of reduced alkaline hematin on addition of Stokes' Reagent.

to make the surgical employment of these bodies dangerous on that account.

The solvent action exerted by the alkaline chlorine disinfectants might be of importance because of danger from secondary hemorrhage in wounds in which they are employed by the Carrel technic, which consists in treating the wound by the constant maintenance of a supply of, and intermittent flushing with, the disinfectant solution. Hence experiments to test the solvent effect of this substance upon blood clot were undertaken (table 8).

Table 8, which shows the effect of chloramine-T in various dilutions, upon blood clot, demonstrates that this agent does not

possess very great solvent powers. This is in accordance with the findings of Austin and Taylor (5) who conclude from their experiments, that chloramine-T solution, even as strong as 2 per cent has no marked erosive effect.

TABLE 8

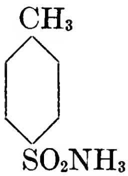
Blood clot with chloramine-T

TUBE	DILUTION	35 MINUTES	3 DAYS	5 DAYS	7 DAYS
Control	0.9 per cent NaCl		Putrefaction, no disintegration		
2	1:20000		Putrefaction, no disintegration		
3	1:10000		Putrefaction, no disintegration		
4	1:2000		Not dissolved	Putrefaction, not dissolved	
5	1:1000		Not dissolved	Putrefaction, not dissolved	
6	1:500	Laking very slight	No odor, some disintegration	Putrefaction, not dissolved	
7	1:250	Laking slight	Slight chlorine odor, some disintegration		Putrefaction
8	Water	Laking slight		Putrefaction, not dissolved	

II. P-TOLUENE SULPHONAMINE AND ITS SODIUM SALT

P-toluene sulphonamine is the parent substance of the chloramines studied.[1] It is a white crystalline odorless body of the following structural formula.

[1] Kindly furnished us by Mr. C. S. Schoefle, Instructor of Organic Chemistry at the University of Michigan.



Its melting point ranges between 136 and 140°C. It is almost tasteless, slightly soluble in water, but dissolves fairly readily in alcohol. It has a feebly acid reaction and dissolves in NaOH, forming a sodium salt, from a solution of which it is reprecipitated by acid.

A saturated solution of p-toluene sulphonamine in normal salt solution was found devoid of any perceptible effect on blood; neither laking, nor change in color or of spectrum being produced by it within twenty-four hours. Infusoria were apparently not harmed by a saturated aqueous solution, their motility being still evident after an exposure of twenty-four hours.

TABLE 9

Toxicity of p-toluene sulphonamine for guinea-pigs on subcutaneous injection

DOSE	NUMBER OF EXPERIMENTS	RESULT
grams per kgm.		
0.5	1	Recovered
1.0	1	Recovered
1.5	2	Both recovered
2.0	4	All died

Two grams per kilogram is evidently a fatal dose for the guinea-pig.

Nevertheless, the substance is toxic to guinea-pigs. A suspension of 2 grams per kilogram in water, injected under the skin of the back, produced, within an hour, evidences of depression. The hind limbs especially were weak; and the animal had difficulty in turning over when placed on its back. Gradually the depression become more marked, the animal remaining lying on its side, the respiration became labored and slow, the corneal reflexes sluggish, and death usually occurred within a few hours, or days. In one animal, occasional clonic spasms were noted. The blood showed a normal spectrum. An animal dying after several days showed a cream colored deposit at the point of

injection. As will be seen from table 9, smaller doses are not fatal, and, aside from transient depression, do not produce marked effects.

We have already called attention to the fact that the intravenous injection of sodium p-toluene sulphonamine does not produce pulmonary edema.

The hemolytic effect of sodium p-toluene sulphonamine upon blood suspension, shown in table 10, is obviously comparable to that of sodium p-toluene sulphonchloramine (chloramine-T) shown in table 5. In view of the negativeness of p-toluene sulphonamine in its laking effect upon the blood suspension, it is reasonable to conclude that the solvent action is due to the alkalinity of the sodium salts.

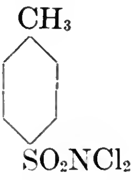
TABLE 10

Blood suspension with sodium p-toluene sulphonamine

DILUTION IN 0.9 PER CENT NaCl	HEMOLYSIS COMPLETE IN	SPECTROSCOPIC EXAMINATION 24 HOURS LATER
1:400	20 minutes	Alkaline hematin
1:800	25 minutes	Alkaline hematin and very faintly oxyhemoglobin
1:1600	35 minutes	Turbid
1:3200	60 minutes	Alkaline hematin and faint oxyhemoglobin
1:6400	One hour, 30 minutes	Oxyhemoglobin
1:12800	3 hours	Oxyhemoglobin
1:25600	No hemolysis	
1:51200	No hemolysis	

III. P-TOLUENE SULPHONDICHLORAMINE (DICHLORAMINE-T).

This substance,[2] which has the following structural formula,



occurs in the form of small colorless crystals, of strong chlorinous odor, almost tasteless, of faint acid reaction, almost insoluble

[2] Kindly furnished us by the Abbott Laboratories of Chicago.

in water (solubility about 1 : 100,000), soluble in alcohol, which it however decomposes. It also decomposes ether and cotton-seed oil. It is quite readily soluble in chloroform and in oil of eucalyptus without evident change. With turpentine it undergoes an explosive reaction, leaving a brown resinous mass, soluble in alcohol, insoluble in water. It dissolves in solution of fixed alkali, with formation of the soluble monochloramine.

$$C_6H_4.CH_3.SO_2.NCl_2 + 2NaOH =$$
$$C_6H_4.CH_3.SO_2.NaNCl + NaO.Cl + H_2O$$

Heating it with strong acids, such as HCl, sets free the chlorine and leaves the sulphonamine. It is a powerful oxidizing agent, readily liberating iodine when treated with potassium iodide.

It is so slightly soluble that its saturated aqueous solution is not disinfectant when poured over a slant culture of staphylococcus. Nor does it kill infusoria when a saturated aqueous solution is mixed with an equal volume of infusorial culture. When, on the other hand, a few crystals of this substance are placed on a slide and these are covered with a drop of infusorial culture, the infusoria in the immediate vicinity of the crystals are killed almost instantly, while further away in the drop, active infusoria can be seen for hours.

The saturated aqueous solution in normal saline is slowly hemolytic and slowly changes hemoglobin to methemoglobin in vitro.

The substance itself is extremely irritating. A little of it, finely powdered and dusted into a rabbit's conjunctiva, produces almost immediate redness; and, within half an hour, extreme chemosis, which lasts for twenty-four to forty-eight hours. There is also a purulent discharge from the eye.

When a suspension of as much as 2 grams of dichloramine-T per kilogram, is injected subcutaneously into a guinea-pig, it apparently produces severe pain, followed by some depression. On the next day, the animal is normal, excepting for a subcutaneous induration at the point of injection. This in the course of a few days, is likely to undergo necrosis, leaving a rather slowly healing ulcer. The low toxicity is evidently in part due to

insolubility; but, as p-toluene sulphonamine which is also quite insoluble, is still capable of producing death from systemic absorption, it is reasonable to assume that the violent inflammatory reaction provoked by the chlorine derivative interferes with the absorption thereof or of its decomposition products from the subcutaneous tissues.

SUMMARY AND DISCUSSION

1. Unicellular animals are promptly killed by very dilute solutions of soluble chloramines.

2. The chloramines are powerful irritants, causing inflammatory edema of the subcutaneous tissue, and even necrosis of the overlying skin on hypodermic injection, inflammation of mucous membranes on local application, and vomiting on oral administration.

3. Chloramine-T depresses the central nervous system in the order of: brain, medulla, spinal cord. This is easily demonstrated in the frog, but it also probably obtains in the higher animals; for, while the depression of the respiratory center which follows depression of the brain leads to death, the fact that asphyxial convulsions generally do not occur points to a depression of the spinal cord. This depression is chiefly due to the p-toluene sulphonamine, though the latter is only about half as toxic as the soluble chlorine derivative, probably because it is much less soluble.

4. On intravenous injection, chloramine-T produces pulmonary edema, probably due to the chlorine in the molecule, as sodium p-toluene sulphonamine given intravenously does not produce such effect. The mechanism of the chloramine-T pulmonary edema is probably the same as that produced by chlorine gas inhalation described and studied by Schäfer (6).

5. The hemolytic power of chloramine-T is due chiefly to its alkalinity, as is shown by the fact that it is also displayed by chlorine-free sodium p-toluene sulphonamine. On the other hand, p-toluene sulphonamine, containing no dissociable alkali, is not hemolytic. Hemoglobin is changed to alkaline hematin by the

first two bodies. Methemoglobin formation due to the chlorine in chloramine-T occurs; but merely to a slight degree, and was only demonstrable in the test tube. Dichloramine-T likewise is slowly hemolytic and slowly changes hemoglobin to methemoglobin in vitro.

REFERENCES

(1) Kastle, J. H., Kaiser, B. C., and Bradley, E.: On the halogen derivatives of the sulphonamines. Am. Chem. Jour., 1896, xviii, 491.

(2) Chattaway, F. D.: Nitrogen derivatives of sulphonamides. Jour. of the Chem. Soc., London, 1905, lxxxvii, (i), 145.

(3) Dakin, H. D., Cohen, J. B., and Kenyon, J.: Studies on antiseptics on chloramines. Brit. Med. Jour., January 29, 1916, i, 160.

Dakin, H. D., Cohen, Daufresne, and Kenyon: Proc. of the Royal Soc., 1916, lxxxix, 232.

(4) Taylor, H. D., and Austin, J. H.: Toxicity of certain widely used disinfectants. Jour. Exp. Med., May 1, 1918, xxvii, no. 5, p. 635.

(5) Austin, J. H., and Taylor, H. D.: Behavior of hypochlorite and of chloramine-T solutions in contact with necrotic and normal tissues in vivo, Jour. Exp. Med., xxvii, no. 5, May 1, 1918, p. 627.

(6) Schäfer, Edward: On the immediate effects of the inhalation of chlorine gas. Brit. Med. Jour., 1915, ii, 245.

HISTAMINE AND PITUITARY EXTRACT

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From the Pharmacological Laboratory, Cambridge

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Abel and Kubota in a recent publication (1) show an interesting parallelism between histamine (and various extracts likely to contain this or similar amines) on the one hand and extracts of the pituitary gland on the other hand, in their effects on the blood-pressure (a) of cats and dogs (b) of rabbits, and on the isolated uterus of the guinea-pig. They form the conclusion that "histamine is the plain-muscle-stimulating and depressor constituent of the posterior lobe of the pituitary gland. The physiological and chemical evidence in favour of the identity of the two principles coincide at every point."

Such a conclusion would doubtless be justified if it could be shown that the two principles produce the same effect under like conditions on all forms of plain muscle. Failing such demonstration or if it can be shown that any form of plain muscle reacts differently to the two principles, this conclusion would appear to be unjustifiable. The plain muscle of the guinea-pig's uterus undoubtedly reacts in the same way to both principles—by a notable increase in tonus with the initiation or increase of movements in a state of raised tonus.

Recent observations in another connection have shown me that on certain tracts of plain muscle the two principles may produce totally dissimilar effects. If for example the uterus of the mouse is suspended as an isolated organ and treated with histamine, one finds that it responds by relaxation, a response similar to that which the guinea-pig's uterus makes to Adrenalin: whilst pituitary extract applied under the same conditions produces an increase in tonus. Figure 1 shows such responses: the active

principles used in this case were the Ergamine phosphate of Burroughs Wellcome Company and Parke Davis' Pituitrin. The concentrations which produced these effects were the minimal concentrations which sufficed to produce any noteworthy effect on this organ. In the case of the mouse and rat, plain muscle in general shows a remarkable tolerance to histamine, the concentration used (found to be the minimal effective strength) in this instance was approximately 1–1000 (as compared with a concentration of about 1–10,000,000 in the case of the guinea-pig's uterus).

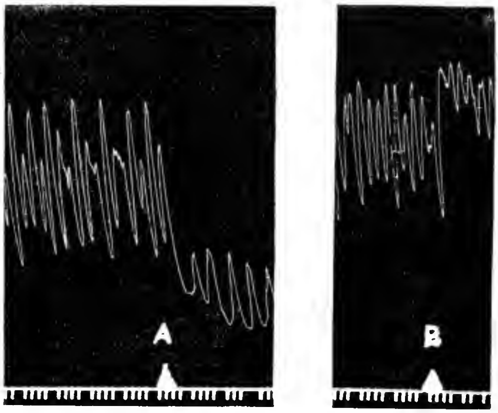

Fig. 1. Isolated Uterus of Cage-Bred Mouse Suspended in Ringer-Locke Solution

Upstroke-contraction. At *A* histamine was added to the bath and at *B* pituitrin. In each case the concentration of the drug was approximately the minimal effective concentration to produce any effect. Tracing reads from left to right.

This is no isolated observation: similar aberrant responses are seen with histamine and pituitary extracts on other tracts of plain muscle not so frequently used for routine laboratory purposes as is the uterus of the guinea-pig. Waddell (2) indeed has shown that pituitary extract normally has no action on the vas deferens of all ordinary laboratory animals, whilst Guggenheim (3) has shown that histamine relaxes the uterus of the rat. I have repeated and confirmed these results and at the same time

have found that on these plain muscle organs pituitary extracts and histamine do not always produce identical effects. It is my hope to make further publication of this work in the near future.

The uterus of the mouse then affords an example of a tract of plain muscle which reacts in one way to histamine and in a diametrically opposite way to pituitary extracts. These results do not appear to bear out the hypothesis advanced by Abel that the plain-muscle-stimulating principles of histamine and pituitary extract are identical.

REFERENCES

(1) Abel and Kubota: This Journal, 1919, xiii, 243.
(2) Waddell: This Journal, 1916, viii, 551.
(3) Guggenheim: Therap. Monatsch., 1912, xxvi, 795.

HISTAMINE AND PITUITARY EXTRACT

JOHN J. ABEL and D. I. MACHT

From the Pharmacological Laboratory of the Johns Hopkins University

In 1912 M. Guggenheim (1) in a paper on the action of para-Oxyphenylethylamine, one of the constituents of ergot, extended his observations to similarly acting substances, and took occasion to compare the action of pituitary extract on the rat's uterus with the action of histamine (β-Iminazolylethylamine) on the same organ. This investigator found that pituitary extract (the Pituglandol of the Hoffmann-La Roche Company) and histamine acted in a diametrically opposite manner upon the rat's uterus, the first mentioned agent stimulating it to contract and producing a great increase in tonus, while the latter failed entirely to cause contractions, inducing instead a marked relaxation and reduction of tonus.

Douglas Cow (2) as will be seen from a perusal of his paper, concludes that the uterus of the mouse likewise affords an example of a tract of plain muscle which reacts in one way to pituitary extracts and in a diametrically opposite way to histamine. The two principles, he says, "may produce totally dissimilar effects." "If, for example, the uterus of the mouse is suspended as an isolated organ and treated with histamine, one finds that it responds by relaxation, a response similar to that which the guinea-pig's uterus makes to Adrenalin: whilst pituitary extract applied under the same conditions produces an increase in tonus." He gives a figure to show the difference in response of the uterus of the mouse to the two agents.

The statements of the authors just cited are quite contrary to the opinions expressed by Abel and Kubota (3), who stated that histamine is the depressor substance and plain muscle stimulant of the posterior lobe of the pituitary gland. Abel and Kubota were fully aware of Guggenheim's work while carrying on their

own researches, and before publishing their paper they performed a number of preliminary experiments in which the effects of pituitary extracts and of histamine on the uterus of the rat were compared. Abel and Kubota did not, however, complete their investigation on the effects of the two products on the isolated uterus of the rat, hoping to examine the subject more carefully at a later date. The fact that Cow has, seven years later, expressed an opinion exactly like that of Guggenheim has induced us to take up the subject at this time.

A glance at the tracings in Cow's paper would appear to overthrow the work of Abel and Kubota. It is a very simple matter, however, to show wherein this investigator is at fault. When he employs any of the pituitary liquids or extracts now used in medical practice, he makes use of a very weak solution of the uterine stimulant. In terms of histamine (assuming for a moment that the uterine stimulant of pituitary liquids is either identical with or at least of equal power with histamine) these pituitary preparations contain only from 1 to 3 mgm. in the ampoule of 1 cc. Investigators who have occasion to use one of the salts of histamine, generally make up a fairly strong solution to start with, and it may happen that the very first portion of the drug which is added to the bath in which the uterus is suspended, either has no marked effect one way or the other upon the organ, or else immediately lowers its tonus, so that relaxation sets in and the lever takes a downward sweep. But if this solution of histamine which has induced relaxation of the uterus is much diluted, and only a small part of the diluted solution is added to the testing chamber, it will be found that the virgin uterus of the mouse will contract vigorously, and will now behave exactly as if a little of a diluted pituitary solution had been employed. In a word, if the solution of histamine hydrochloride or of histamine acid phosphate (Ergamine Acid Phosphate, Burroughs, Wellcome and Company) is added in such small quantities that the testing chamber contains approximately 1–1,000,000 or 1–2,000,000 of the drug, as the case may be, a very pronounced contraction of the uterus with increase of tonus will result. In proof of this statement we refer to figures 1, 2 and 3.

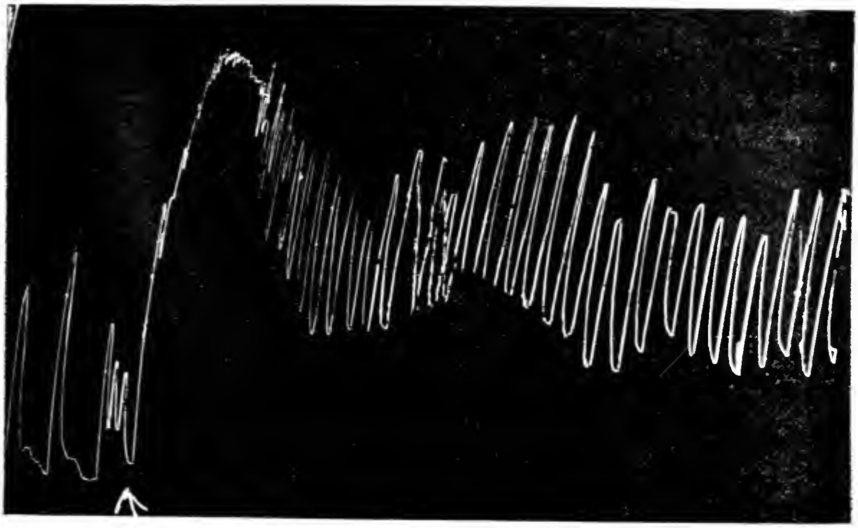

FIG. 1. ONE HORN OF VIRGIN UTERUS OF MOUSE IN 25 CC. OF LOCKE'S SOLUTION

At ↑ $\frac{1}{28}$ cc. of a diluted Pituitrin solution (1:5) obstetrical, Parke, Davis and Company (chloretone having been removed by evaporation on the water bath) was added. On the assumption that the 1 cc. ampoule contained the equivalent of 3 mgm. of histamine hydrochloride the concentration of pituitary substance acting on the uterus would be 1:1,170,000.

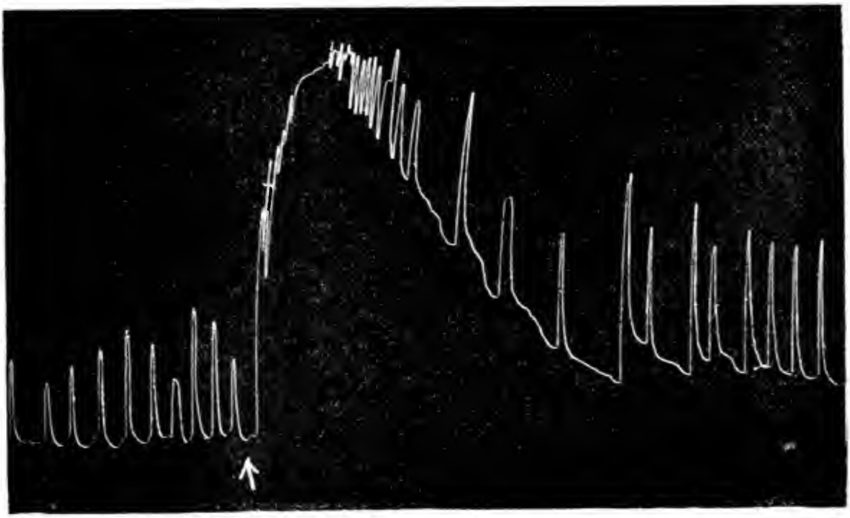

FIG. 2. OTHER HORN OF VIRGIN UTERUS OF MOUSE USED IN FIGURE 1

At ↑ $\frac{5}{28}$ cc. of a solution containing $\frac{12}{100}$ mgm. of histamine hydrochloride in the cubic centimeter was added to the 25 cc. of Locke's solution in the chamber; concentration of histamine 1:1,170,000.

From this high dilution, one can work one's way backward, so to speak, by increasing the strength of the solution until there is practically no external response to the drug. But to a further increase in the strength of the solution the uterus of the mouse, or guinea-pig responds with relaxation and entire loss of tonus. Cow very evidently *began* his tests with histamine on the uterus of the mouse by employing a paralyzing concentration of the drug at the very start, inasmuch as he expressly states that "the minimal effective concentration was approximately 1–1000,

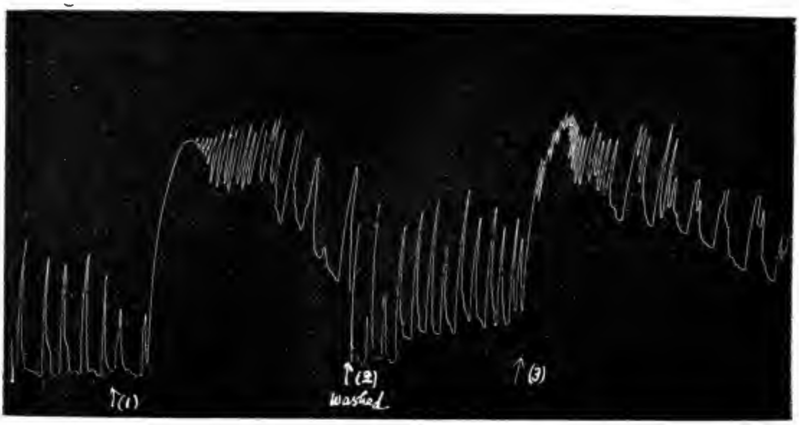

FIG. 3. VIRGIN UTERUS OF THE MOUSE

At *1* $\frac{1}{14}$ cc. of "Pituitrin." (obstetrical) Parke, Davis and Company, taken directly from the ampoule was added to the chamber containing 25 cc. of Locke's solution. On the assumption that the ampoule contained an amount of active substance equal to 3 mgm. of histamine hydrochloride, the concentration in the bath would be 1:583,330. At *2* the pituitrin was removed by washing out the chamber three times with Locke's solution. At *3* $\frac{3}{14}$ cc. of a solution of histamine hydrochloride containing $\frac{12}{100}$ mgm. in the cubic centimeter was added to the bath. Concentration in chamber 1:972,200.

as compared with a concentration of about 1–10,000,000 in the case of the guinea-pig's uterus." As is abundantly shown in our tracings, it is only necessary to use a concentration of 1–500,000 or 1–1,200,000 to obtain the most vigorous contractions of the uterus of the mouse. The action of a moderately

strong solution of Ergamine Phosphate on the uterus of the rabbit is shown in figure 4.

In comparing the action of the dihydrochloride with the acid phosphate of histamine, one naturally bears in mind that the relative amount of histamine in the two salts is in the ratio of 1:1.7.

Fig. 4. Virgin Uterus of Rabbit Showing Stimulating Action of Ergamine Acid Phosphate in the Concentration of 1:625,000

SIMILARITY IN THE ACTION UPON THE UTERUS OF MORE CONCENTRATED PITUITARY EXTRACTS AND HIGHER CONCENTRATIONS OF HISTAMINE

In figures 1, 2 and 3 we have shown that when weak pituitary solutions and weak histamine solutions are employed, there is absolutely no difference whatever in the response of the uterus of the mouse to the two solutions when comparable amounts of the two drugs are employed. Uterine tracings entirely identical in appearance are easily prepared.

How does the matter stand when more concentrated solutions of the two agents are employed? It will be remembered that in Cow's tracings we are comparing the paralyzing strength of 1–1000 Ergamine Acid Phosphate with a small amount of a weak extract of the pituitary gland. Suppose now that the contents of a number of ampoules of any of the pituitary liquids upon the

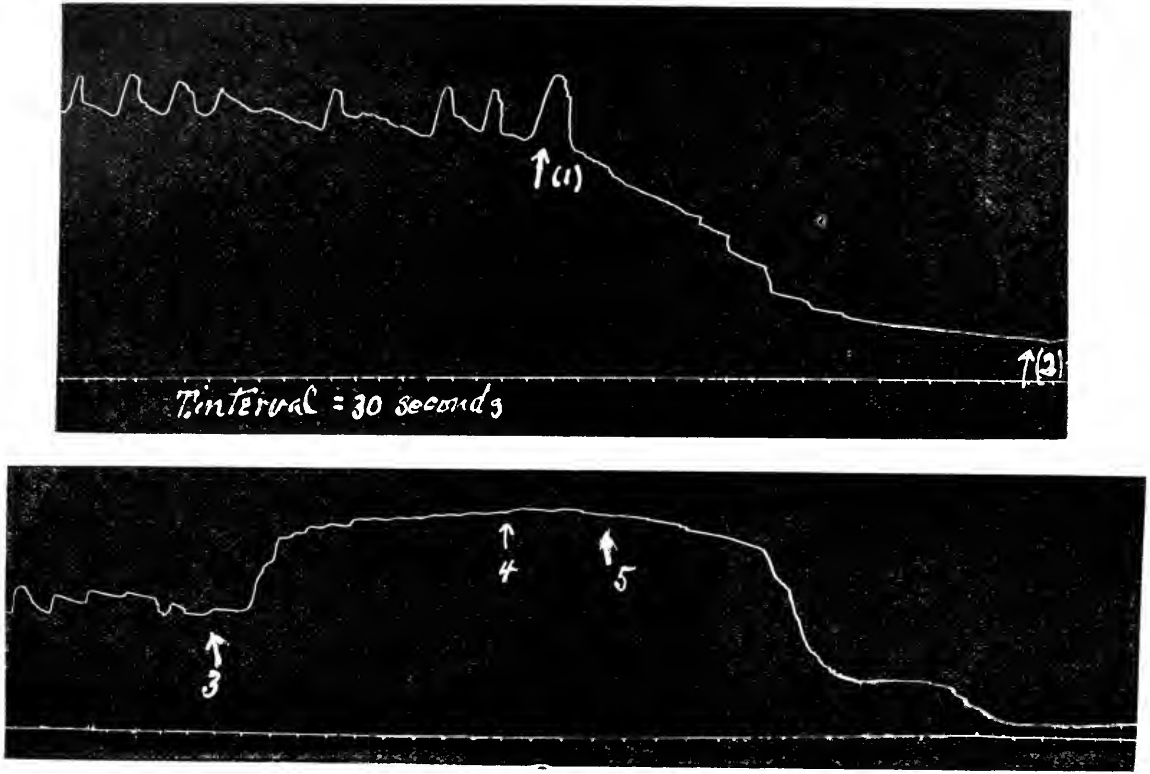

Fig. 5. Shows Paralyzing Action of Large Doses of Pituitary Extract

Left horn of virgin uterus of the mouse. At *1* the neutralized residue of 6 cc. of "Pituitrin" (obstetrical), dissolved in 2 cc. of water was added to the chamber containing 23 cc. of Locke's solution. On the assumption that each cubic centimeter of the "Pituitrin" contains the equivalent of 3 mgm. of histamine hydrochloride, the concentration of active principle in the chamber would be 1:1333. At *2* the Pituitrin was removed by changing the Locke's solution three times and after an interval of an hour or more the uterus again beats spontaneously. At *3* one-twentieth of an ampoule of Pituitrin was added; at *4* the drum was halted for ten minutes, the uterus remaining in tonic contraction; at *5* the neutralized residue of 3 cc. of "Pituitrin" was added to the bath.

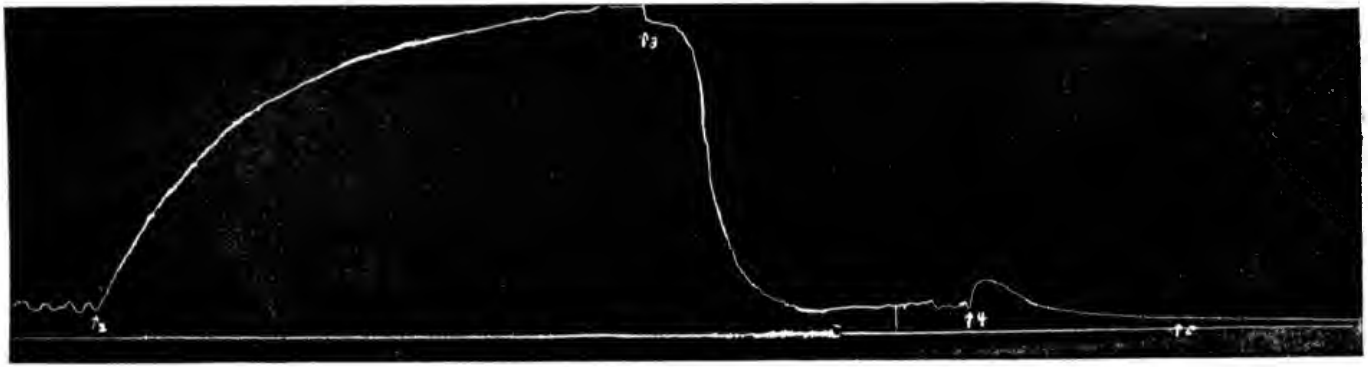

FIG. 6. SHOWING STIMULATION OF THE VIRGIN GUINEA-PIG'S UTERUS BY A SMALL AMOUNT OF PITUITARY LIQUID (ARMOUR AND COMPANY) FOLLOWED BY COMPLETE RELAXATION AND PARALYSIS ON THE ADDITION OF LARGER AMOUNTS OF THE LIQUID

Because of vigorous normal contractions, the uterus was heavily weighted, and consequently was stretched nearly to its maximum, and hence when poisoned it could not relax much below its original level of vibration. At *2* $\frac{1}{500}$ cc. of an Armour's ampoule; concentration 1:4,166,666; at *3* Locke's solution changed; at *4* the residue of 6 ampoules (Armour's) neutralized, dissolved in 3 cc. water and added to 22 cc. Locke's solution in the chamber, concentration on the basis of previous estimates 1:1388; at *5* the residue of six ampoules in 3 cc. of water again added. From *2* to *4* the tracing was redrawn in consequence of an error in shellacing.

market are concentrated on the water bath, under an electric fan, almost to dryness, and the concentrated and neutralized[1] solution of this known number of cubic centimeters of pituitary fluid is added to the uterine chamber, what now will be the result? The answer is simple and is given in figures 5 and 6. It will be seen that when a sufficient number of ampoules are used, six or more, as the case may be, the result is entirely like that produced when strong histamine solutions are used, as is shown in figures 7 and 8. It may be noted in passing that the uterine stimulant is not weakened in its action by this rapid evaporation of a pituitary extract on the water bath at a low temperature, as is shown by dissolving the residue and comparing its activity with that of an equal volume of the original extract.

When not too large an amount of a strong solution of either drug is used, the uterus may make a slight attempt at contraction, and then continue beating as though no influence had been brought to bear upon it. On the addition of more of the concentrated solution, however, immediate relaxation, absence of rhythmic contractions and loss of tonus occur. In a word, we can duplicate by means of concentrated pituitary extracts the picture that is produced when strong solutions of histamine are employed.

An interesting fact emerges here. We have found by means of comparative physiological tests that the pituitary liquids now on the market,[2] so far as we have examined them, contain close to 3 mgm. of active uterine stimulant, in terms of histamine, in the cubic centimeter. On the basis of this calculation it will be observed that the amount of pituitary principle required to cause paralysis or relaxation of the uterus of the mouse or guinea-pig corresponds very nicely with the amount of histamine that is necessary to produce paralysis. It would perhaps take more experiments than we have made, and the use of a considerable amount of expensive material, to give entirely exact ratios for this paralytic action; but we have, nevertheless, gone far enough to

[1] Made neutral to litmus.

[2] We speak here of the pituitary liquid of Armour and Company, and of the Pituitrin (obstetrical) of Parke, Davis and Company.

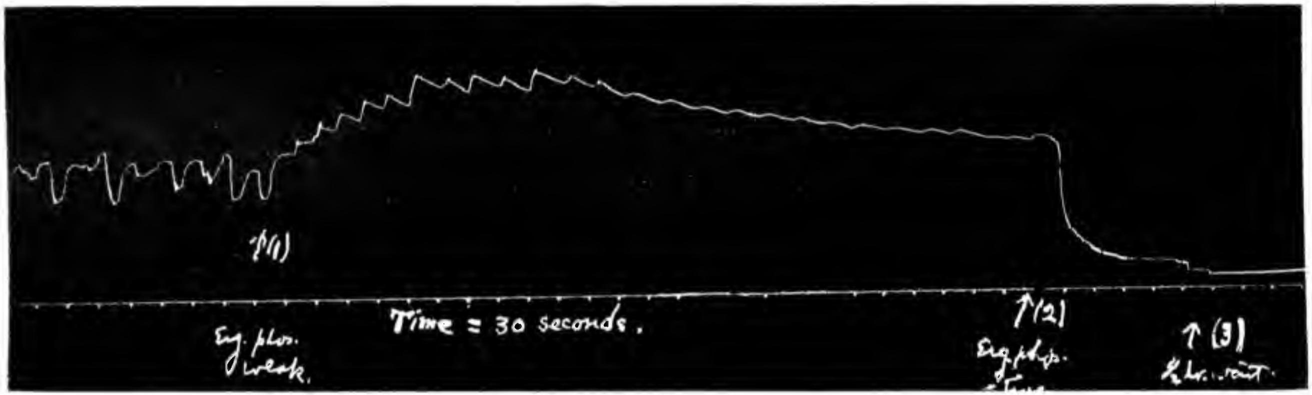

FIG. 7. RIGHT HORN OF VIRGIN UTERUS OF MOUSE, THE LEFT HORN OF WHICH WAS USED FOR TRACING IN FIGURE 5

Shows stimulating action of small amounts of ergamine phosphate followed by the paralyzing action of large doses At 1 $\frac{1}{80}$ mgm. of ergamine phosphate was added to the 25 cc. Locke's solution in the chamber; concentration 1:2,000,000. At 2 added 20 mgm. of ergamine phosphate in 2 cc. water to 23 cc. Locke's fluid in the chamber. Concentration 1:1250. At 3 drum was halted for one-half hour.

Fig. 8. Showing the Stimulating Action of a Small Amount of Ergamine Phosphate (Burroughs, Wellcome and Company) on the Virgin Uterus of the Guinea-Pig, and the Paralyzing Action of Larger Amounts of the Drug

To be compared with figure 6 in which the paralyzing action of Armour's pituitary liquid is shown. At *1* $\frac{1}{125}$ ergamine phosphate; concentration 1: 3,125,000. At *2* 3 mgm. ergamine phosphate; at *3* 5 mgm. ergamine phosphate, at *4* 15 mgm. ergamine phosphate. Complete relaxation. Fluid in the chamber now 27.3 cc.; concentration now 1: 1087.

convince ourselves that a very exact comparison can here be made. In a word, we start with dilute solutions of histamine and pituitary extract which are equally effective in stimulating the uterus to contract and assume for the moment that the latter solution contains the *same* amount of histamine as the former. As shown in our tracings, two such dilute solutions actually stimulate the uterus to the same extent. On the basis of this calculation, we now approach the strong solutions necessary to paralyze the uterus, and here again the parallel holds—solutions of approximately the *same* strength, on the basis of the assumption above made, paralyze the organ to the same extent. This is clearly demonstrated in figures 5 and 7. In figure 5, the tracing shows that the left horn of the virgin uterus of the mouse was paralyzed by a concentration of pituitary active principle of 1 : 1333. In figure 7, the concentration of ergamine phosphate which completely paralyzed the right horn of the same uterus proved to be 1: 1250. Equally depressing are strong solutions of the two drugs for the guinea-pig's uterus, as is shown in figures 6 and 8, in which two different uteri of this animal are used. Here the concentration of ergamine phosphate necessary to paralyze is 1: 1087, as against a pituitary concentration of 1: 1388. That the correspondence in the concentrations needed to paralyze the two guinea-pigs' uteri is not quite as close as in the experiments described under figures 5 and 7 is understood when it is noted that in experiment 8 the ergamine phosphate was added to the chamber in three separate portions. We feel certain that it would not have required the addition of more than 20 mgm. of the drug at (2) to induce the paralysis which actually occurred at (4). On this assumption the concentration would work out to be 1: 1250 for the ergamine phosphate, as against 1: 1388 for the pituitary liquid. But it will be noted that the pituitary solution was not quite strong enough to paralyze the uterus at once; otherwise the correspondence might have been closer.

This striking parallelism does not at all prove that histamine is in reality the uterine stimulant of the posterior lobe of the hypophysis, since a very different principle from histamine might show a similar ratio between a stimulating and a paralyzing

strength of solution. The correspondence is, nevertheless, striking and of great interest in this connection.

It has been shown then that the guinea-pig's uterus is as easily paralyzed as is that of the mouse by large doses of histamine and by concentrated pituitary extracts, and it will be of interest to learn to what extent this fact obtains for other tracts of plain muscle that are stimulated by minute quantities of the two agents.

In this connection we may state that the paralyzing action of concentrated pituitary extracts cannot be due to tissue elements such as amino-acids, salts or albumoses present in such extracts. The effect of such substances in the quantities present, is rather of a stimulant than a depressant nature. The total amount of both organic and inorganic matter present in the cubic centimeter of the "Pituitrin" (obstetrical) used by us was 0.0091 gram. In order to test the action on the uterus of the extraneous elements in Pituitrin, an aqueous extract of the anterior lobe of the hypophysis was prepared and the amount of organic and inorganic matter in the cubic centimeter of the extract determined. On adding this extract to the uterine chamber in amounts as high as 166 mgm., as against 55 mgm. or thereabouts which always caused paralysis when Pituitary residues were employed, only stimulation of the guinea-pig's uterus was observed to take place and the effect was about equal to that induced by a pituitary extract 1: 10,000,000. We cannot avoid the conclusion therefore that the paralyzing action on the uterus of an excess of pituitary extract (6 cc. or more) as described by us is due entirely to the presence in *excess* of that agent which acts as a powerful stimulant when only small quantities of the extract are used.

A later communication from this laboratory will deal with the action of pituitary extracts on the vas deferens. Cow cites Waddell as stating that pituitary extract normally has no action on the vas deferens of the ordinary laboratory animals. Macht and Matsumoto (4) have observed in this laboratory that while the vas deferens is but little responsive to small doses of pituitary extract or histamine, it contracts however when stronger solutions of the two drugs are applied.

Our investigation on the comparative effects of pituitary extracts and of histamine on the uterus of the rat will be deferred until we have made further chemical studies of the pituitary gland. At the moment, we concede to Guggenheim that the uterus of the rat behaves differently in its response to the two agents—pituitary extracts and histamine—from the uterus of the mouse, guinea-pig and rabbit. While histamine hydrochloride in weak solutions, 1 to several millions, may cause only a slight increase in the tonicity of the rat's uterus, a correspondingly weak pituitary extract (on the basis of the calculations already given) may induce a much more marked contraction of the uterus. Stronger solutions of histamine as 1: 100,000 will cause relaxation or even paralysis, while pituitary extracts of this strength induce very good though not maximal tonic contractions. There is here then no such correspondence between the stimulating and the paralyzing doses of the two agents as is observed in the case of the mouse and guinea-pig. And further the dose of pituitary extract required to paralyze the uterus of the rat is very large; in fact it has not yet been determined by us. At the moment of writing we have failed entirely to cause relaxation of the tetanic state into which the neutralized residue of two ampoules had thrown a small piece (one-third) of one horn of a rat's uterus, even by the use of the contents of 16 ampoules (Parke, Davis and Company and Armour and Company) added to the chamber (25 cc. Tyrode's solution) in the course of half an hour. The experiment continued from 2.30 p.m., when the tetanic state was induced, until 5.15 p.m. at which time the uterus was still as firmly contracted as at the 2.30, the lever having fallen hardly at all below the tetanic level in all this time.

Various deductions may be drawn from the above as yet incomplete studies of the comparative action of pituitary extracts and salts of histamine on the rat's uterus. When we compare the effects of a mixture of substances (pituitary extract, containing a depressor and a pressor substance) with the effects of a single chemical individual, as histamine, on a variety of animal tissues, we must not be surprised if we meet with anomalous reactions. Let us suppose that the depressor substance and uterine

stimulant of Abel and Kubota is present in two forms, (a) as a histamine derivative or compound (b) as a free histamine salt, the two forms being in equilibrium with each other (an hypothesis which is now being tested out in this laboratory) and that the uterus of the rat reacts differently to (a) and (b) while that of the mouse, guinea-pig and rabbit react in the same manner to both. These and other possibilities must be put to the test before we can conclude that the discrepancies noted in relation to the rat's uterus prove that histamine or a histamine derivative is *not* the plain muscle stimulant of the pituitary gland. As already intimated we are now engaged in the study of these questions. The writers have lately obtained conclusive evidence, in experiments which can not be here detailed, that there is present in the posterior lobe a substance which readily yields histamine on treatment with acids and the confidently believe that this precursor of histamine will exhibit, when isolated, the action of histamine to an intense degree.

But whatever may be the outcome of future studies as to the behavior of the rat's uterus in response to pituitary extracts and histamine, we believe that we have shown that Cow's contention the uterus of the mouse reacts to histamine and in a diametrically opposite way to pituitary extracts is without foundation.

SUMMARY

1. The uterus of the mouse and of the guinea pig react in the same way toward pituitary extracts and salts of histamine, in the sense that both tracts of plain muscle respond to small and presumably comparable doses by contractions and increase of tonus, the uterus of the guinea-pig being the more sensitive of the two to minute doses of the two agents.

2. Both tracts of plain muscle are easily paralyzed by comparable doses of pituitary extracts and of histamine salts.

3. An interesting parallelism therefore is shown to exist in the manner in which comparably weak solutions of the two agents stimulate both tracts of plain muscle to an equal degree, while equally strong solutions of the two agents paralyze both tracts.

4. The rat's uterus presents certain anomalies in its reaction to both pituitary extracts and histamine as compared with the reaction of the uterus of the mouse or guinea pig to these agents, and these require further study, more especially from the chemical point of view, before definite conclusions can be drawn in regard to the significance of these anomalous reactions.

5. The hypothesis that histamine may be present in the posterior lobe of the pituitary gland in two forms (a) as a histamine compound (b) as free histamine in equilibrium with the compound is now being put to the test in this laboratory.

REFERENCES

(1) Guggenheim, M.: Therapeutische Monatshefte, 1912, xxvi, 795.
(2) Cow, Douglas: Jour. Pharmacol. Exp. Therap., 1919, xiv, 275.
(3) Abel, J. J., and Kubota, S.: Jour. Pharmacol. Exp. Therap., 1919, xiii, 243.
(4) See Matsumoto, S., and Macht, D. I.: Jour. Urology, iii, No. 2, April, 1919, p. 70, for statement that the vas deferens of ordinary laboratory animals responds with contractions only to large doses of gland extracts, the pituitary included. Recently these investigators have found that the vas deferens also responds with contractions to large doses of histamine. They will report their findings in a forthcoming number of the Journal of Urology.

SOME OBSERVATIONS ON THE ACTIVE PRINCIPLES OF THE PITUITARY GLAND

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It is a matter of common knowledge that aqueous extracts of the infundibular portion of the pituitary gland contain certain constituents which exert powerful physiological actions on the blood pressure and on plain muscle.

In spite of a vast amount of work on the subject the chemical nature of these substances is as yet obscure.

Some preliminary experiments have recently been made in this laboratory with the ultimate intention of attempting to isolate the active principles and a few useful facts have been established.

Following common practice the dried and powdered infundibulum[1] was treated with hot water with the addition of a few drops of acetic acid to coagulate the protein. On extracting the dry powder with one hundred times its weight of acidulated water and filtering, a perfectly clear solution was obtained. A surprisingly large amount of material—about 20 per cent by weight of the dry powder—was found in this "1 per cent extract."

A partial purification of the "1 per cent extract" can be effected by treatment with colloidal ferric hydroxide and subsequent filtration, without the loss of more than a trace of the active principles. About 16 per cent of the solid matter is removed from the "1 per cent extract" by this procedure. The fact that the active principles are not precipitated by colloidal ferric hydroxide is somewhat surprising, for Guggenheim (1914) reports that they are readily adsorbed by lead sulphide and even by talcum. On the other hand Aldrich (1908) successfully effects a similar purification by means of uranium acetate.

[1] This material was furnished by Messrs. Burroughs, Wellcome and Company.

The filtrate, after treatment with colloidal ferric hydroxide, displays a faint acidity to litmus, due to hydrochloric acid derived from ferric chloride contained in the colloidal solution.

On submitting this filtrate to continuous extraction with butyl alcohol at reduced pressure according to the recent suggestion of Dakin (1918) the active principles pass into the butyl alcohol. Under the experimental conditions adopted, extraction for twelve to fifteen hours suffices to remove completely and quantitatively the uterine stimulant. Not so the pressor principle, of which only about 50 per cent is found in the butyl alcohol extract, while 40 per cent is still detectable in the aqueous layer. It seems that a slight destruction of this second substance occurs during the process of extraction.

This observation, then, affords definite evidence in favor of the view that the two substances are distinct chemical individuals, of which the uterine stimulant is the simpler, although it seems probable that they are very similar in chemical structure. It is interesting to note that Engeland and Kutscher (1912) report that they succeeded in obtaining a substance which stimulated the isolated uterus but had no action on the blood pressure. The work of Schafer and Vincent (1899), Osborne and Vincent (1899) and others all leads to the conclusion that the various physiological effects of pituitary extracts are due to a variety of active substances.

On evaporation of the butyl alcohol extract a crystalline residue is obtained which is extremely soluble in water with an acid reaction to litmus. It contains the active constituents (probably as hydrochlorides) together with contaminating substances (e.g., amino-acids) extractable by butyl alcohol.

About 60 per cent of the solid matter left in the filtrate after the colloidal ferric hydroxide treatment is extracted by butyl alcohol. Thus about 50 per cent of the total solid matter of the original "1 per cent extract" can be eliminated without appreciable loss of the uterine stimulant.

Butyl alcohol also extracts this principle readily from a solution rendered alkaline by the addition of sodium carbonate.

Exposure of "1 per cent extract" to ultraviolet light results in rapid inactivation of the uterine stimulant.

Just as these preliminary experiments were concluded a paper was published by Abel and Kubota (1919) in which they ascribe the action of extracts of the pituitary gland on plain muscle simply to the presence of histâmine, and by subjecting a large quantity (a pound) of dried substance from the *whole* pituitary gland to a rather complicated series of chemical operations they actually succeed in isolating and identifying a very small quantity of histamine (18 mgm. of the dipicrate).

In view of the importance of Abel and Kubota's statements it seemed necessary definitely to settle the question as to the possible identity of the uterine stimulant of the infundibular lobe with histamine.

Already observations have been published which militate against this view. Notably Guggenheim (1914), in describing the sensitiveness of "pituglandol" to alkali, reports an experiment showing that under conditions which destroy both the pressor and muscle-stimulating activities of "pituglandol" the action of histamine on the blood pressure remains unaffected. Guggenheim, on account of the simultaneous destruction of both pressor and oxytocic properties of "pituglandol" by dilute alkali, regards the two effects as being due to one and the same substance, and therefore, presumably, did not make a similar comparison of the actions of alkali-treated histamine and "pituglandol" on the isolated uterus.

This experiment has therefore been performed and has demonstrated that a 0.036 per cent solution of the purified active residue obtained by butyl alcohol extraction is completely inactivated by standing at room temperature for two hours with an equal volume of 2n sodium hydroxide, whereas a 0.007 per cent solution of histamine phosphate, when treated with sodium hydroxide in precisely the same manner, retains to the full its action on the uterus of the guinea-pig.

It had previously been observed that the active principles of the pituitary gland were rapidly decomposed by the action of trypsin. Accordingly a 0.01 per cent solution of the purified active material in 0.1 per cent sodium carbonate, and a 0.0022 per cent solution of histamine phosphate in 0.1 per cent sodium

carbonate were subjected to the action of the same trypsin preparation. In half an hour the oxytocic action of the pituitary principle had almost completely disappeared, while that of the histamine remained absolutely unimpaired.

Since Abel and Kubota make use of the solubility of the free base in hot chloroform in isolating histamine, the solubility of the uterine stimulant in this solvent was investigated, in order to ascertain whether it behaved similarly to histamine. "One per cent extract," after treatment with ferric hydroxide, and a solution of histamine phosphate were rendered alkaline by the addition of sodium carbonate solution and evaporated to dryness over sulphuric acid in a vacuum desiccator. The dry residues were then extracted six times with boiling chloroform under identical conditions. Over two-thirds of the histamine was extracted, while not more than a trace of the oxytocic principle passed into solution in the chloroform.

It has also been noted that the extraction of histamine hydrochloride from aqueous solution by butyl alcohol proceeds only very slowly as compared with that of uterine stimulant from acid (HCl) solution. Under conditions which result in the complete extraction of this latter substance histamine hydrochloride is only extracted to the extent of one-twelfth of the amount taken.

These experiments prove decisively that the activity on the uterus of extracts of the posterior lobe of the pituitary gland is due not to histamine but to some other, as yet unidentified, chemical substance.

It may be definitely stated, further, not only that the uterine stimulant is not histamine, but also that there is no detectable quantity of histamine present in extracts of the infundibular material. For if the "1 per cent extract" had contained small traces of histamine the inactivation by alkali would have been incomplete, and, moreover, the chloroform extract of the dry alkaline residue would have displayed some action on the uterus.

The fact remains, however, that Abel and Kubota have undoubtedly isolated histamine from pituitary material, although the amount actually obtained was very small. It is worthy of

note that they used the *whole* pituitary gland, while in my experiments only the infundibular portion was employed. It would be of considerable interest if it were found that histamine actually occurred in the anterior lobe of the pituitary body. At the same time the possibility of the production of small amounts of histamine by slight putrefactive changes during the collection and subsequent drying of tissues generally should not be overlooked. There is the further possibility that the original dried material was free from histamine, and that traces of this base were produced in the course of the chemical treatment to which Abel and Kubota subjected the material in their process of extraction. In the same paper they give preliminary evidence of the formation of histamine from proteins during gentle acid hydrolysis.

I desire to acknowledge my indebtedness to Dr. H. H. Dale for much valuable help and advice during the performance of these experiments.

EXPERIMENTAL

Preparation of "1 per cent extract"

The dry, powdered infundibular material was heated with one hundred times its weight of distilled water in a boiling water bath for ten minutes. Two to three drops of n/2 acetic acid for every 10 cc. of water taken were added, and the heating continued for another ten minutes. The solution was then allowed to cool and filtered. Filtration from the precipitated protein proceeded without difficulty, a clear, colorless solution being obtained.

Treatment with colloidal ferric hydroxide

Five cubic centimeters of the "1 per cent extract" were taken and colloidal ferric hydroxide solution added drop by drop from a burette. After each addition the precipitate was allowed to settle. The supernatant liquid remained perfectly clear until excess of ferric hydroxide had been added when it assumed a brown tint. It is easy thus to "titrate" the "1 per cent extract" with colloidal ferric hydroxide. Using a solution containing ap-

proximately 5 per cent Fe_2O_3 it was found that after the addition of 0.2 cc. to 5 cc. "1 per cent extract" the supernatant liquid was colorless, while it was tinged brown by a further quantity of 0.05 cc. The "titrated" 5 cc. were then mixed with the main bulk of the "1 per cent extract" and the requisite multiple of 0.2 cc. colloidal ferric hydroxide added. The solution was then heated for two minutes in a boiling water bath, cooled and filtered. A water-clear liquid was obtained.

Five cubic centimeters "1 per cent extract" were evaporated to dryness in a vacuum desiccator over sulphuric acid. The residue weighed 0.0110 gram; "1 per cent extract" therefore contained 0.22 per cent solid matter.

The remainder (15 cc.) of this "1 per cent extract" was treated with 0.6 cc. colloidal ferric hydroxide solution and 5 cc. of the filtrate were evaporated to dryness as above. The residue weighed 0.0089 gram. The filtrate therefore contained 0.18 per cent solid matter.

Extraction of aqueous solutions by butyl alcohol at reduced pressure

A boiling flask (*A*), of about 55 cc. capacity, is provided with a sealed-in capillary bubbling tube (*B*) and a side tube (*C*). The flask is attached to a wide glass tube which passes through a condenser as shown. A T-piece is fused on to the other end of this tube. The upper end of this T-piece is attached to the vacuum pump. The lower end is fitted with a rubber stopper and this carries a wide tube (*D*) of about 40 cc. capacity. Inside this tube a narrower one (*E*) with a perforated bulb at the lower end is placed. The side tubes of (*A*) and (*D*) are connected by means of a slightly constricted tube (*F*). Butyl alcohol is placed in (*A*) and the liquid to be extracted in (*D*). With a pressure of 10 to 15 mm. and (*A*) in a water bath at 45 to 50°C. the butyl alcohol boils and passes through the liquid to be extracted. It returns to (*A*) through the side tubes, the constriction in (*F*) forming a liquid lock which prevents the escape of butyl alcohol vapor by that route.

All the extractions recorded in this paper were made in this apparatus under the conditions mentioned above.

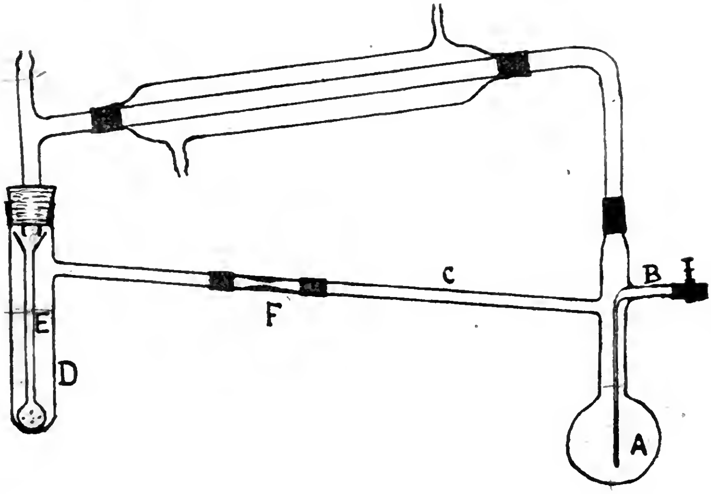

Fig. 1

Extraction of pituitary active principles

1. *From acid* (HCl) *solution.* (See fig. 2.) Thirty cubic centimeters "1 per cent extract" were made and a sample set aside for physiological test (solution a).

The remainder (25 cc.) was treated with colloidal ferric hydroxide and a sample of the filtrate preserved (solution b). The rest of the filtrate (20 cc.) was extracted for twelve hours with butyl alcohol.

The butyl alcohol extract was evaporated to dryness in vacuo, water was added to the residue and then distilled off in vacuo to remove traces of butyl alcohol. This process was repeated and finally the residue was dissolved in water, filtered from traces of greasy matter and made up to 20 cc. (solution c).

The unextracted material in the aqueous layer was treated similarly and finally obtained in solution in 20 cc. water (solution d). This series of solutions was tested on the isolated horn of the guinea-pig's uterus suspended in a 55 cc. bath of Ringer solution, according to the method of Dale and Laidlaw (1912).

The solutions displayed a vey faint acidity to litmus and were neutralized just before testing with a drop of 1 per cent Na_2CO_3 solution. Portions of them were diluted twenty times and used for the tests.

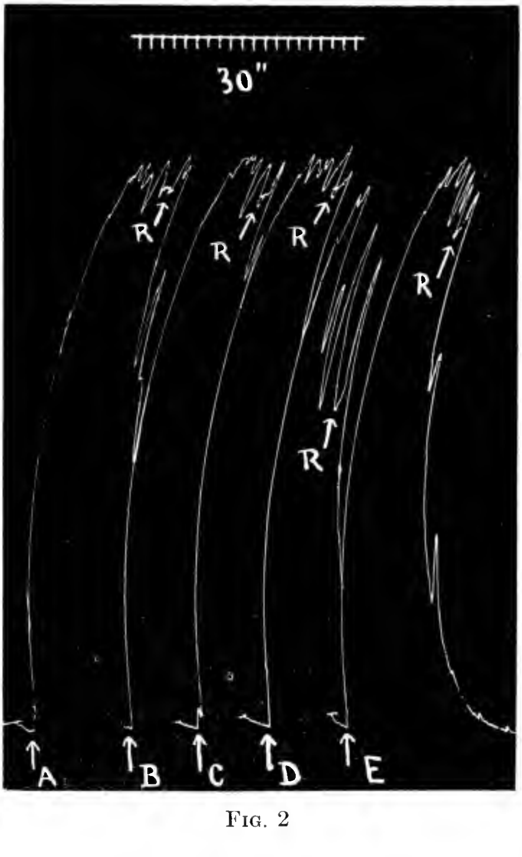

FIG. 2

At *A*, 0.5 cc. diluted solution b was introduced.
At *B*, 0.5 cc. diluted solution a was introduced.
At *C*, 0.55 cc. diluted solution c was introduced.
At *D*, 0.5 cc. undiluted solution d was introduced.
At *E*, 1.0 cc. undiluted solution d was introduced, i.e., the equivalent of 20 cc. of the diluted solutions.

R indicates the points at which the bath was washed out and filled with fresh Ringer.

The tracings show that:

1. No appreciable amount of the uterine stimulant is lost by the colloidal ferric hydroxide treatment.

2. The uterine stimulant is extracted without decomposition by butyl alcohol at reduced pressure.

3. After twelve hours extraction not more than one-fortieth of the uterine stimulant remained unextracted.

Figure 3 shows the distribution of the pressor principle between the butyl alcohol extract and aqueous residue obtained in the above extraction.

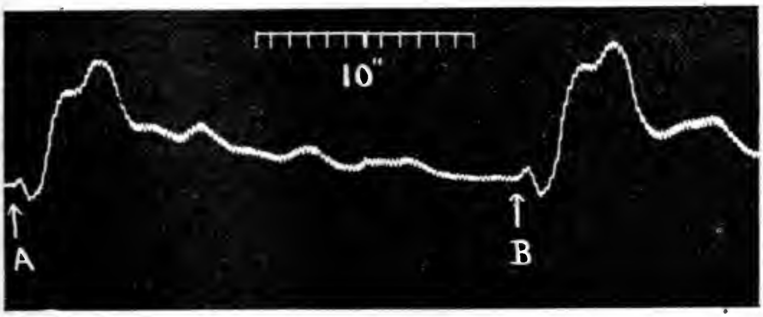

FIG. 3

At *A*, 0.5 cc. solution d was injected intravenously into a decerebrated cat,[2] while at *B*, 0.5 cc. of the solution c was injected. The pressor effects were matched with that produced by solution b, when it was found that solution d had about 40 per cent, and solution c about 50 per cent of the activity of solution b.

Thus, while all but a trace of the uterine stimulant had been extracted by butyl alcohol, not more than 60 per cent of the pressor principle had been removed from the aqueous layer.

In a typical extraction of a solution containing 0.054 gram solid matter 0.034 gram was found in the butyl alcohol extract and 0.0206 gram in the aqueous layer; i.e., 62.3 per cent was extracted.

[2] Dr. Dale kindly performed this experiment.

Extraction of pituitary active principles

2. From alkaline solution. To 25 cc. "1 per cent extract," after the usual treatment with colloidal ferric hydroxide, was added 1 cc. 1 per cent Na_2CO_3. This solution, which was alkaline to litmus, was extracted for fifteen hours. The extract and residue were dissolved in water, neutralized with HCl, made up to the original volume and tested on the uterus in exactly the same way as in the case of the acid extraction (fig. 4).

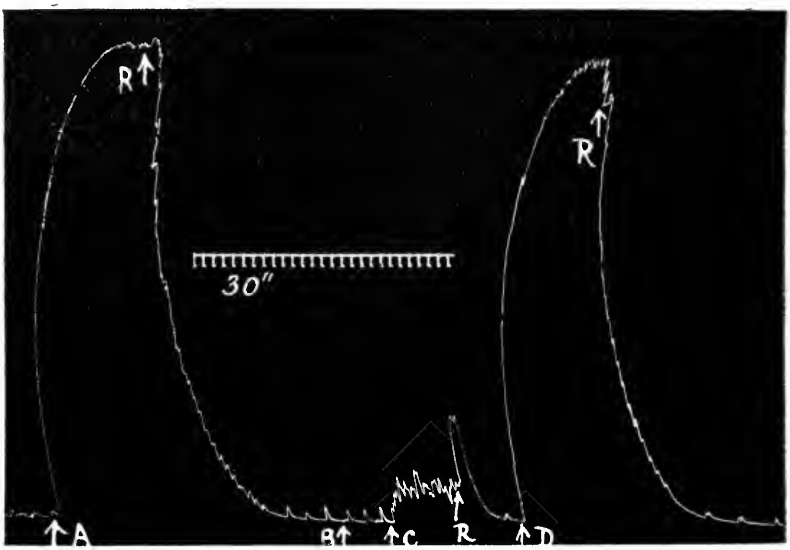

Fig. 4

At *A*, 0.5 cc. of a 1:20 dilution of the original unextracted solution was allowed to act on the uterus.

At *B*, 0.5 cc. of the solution of the residue (diluted 20 times) was added.

At *C*, 2 cc. of the undiluted solution of the residue was added; i.e., the equivalent of 40 cc. of the diluted solutions.

At *D*, 0.5 cc. of the solution of the butyl alcohol extract (diluted 20 times) was added.

Thus it is seen that butyl alcohol extracts completely and without decomposition the uterine stimulant from faintly alkaline solution.

Extraction of histamine and its salts by butyl alcohol

1. Histamine. To 0.0017 gram histamine phosphate, dissolved in 20 cc. water, 1 cc. 1 per cent Na_2CO_3 was added and the solution extracted with butyl alcohol for twelve hours. The butyl alcohol extract and the residue were worked up as in the case of the pituitary experiments, the residue being neutralized with HCl. Both were dissolved in 20 cc. water, and tested on the isolated uterus of the guinea-pig (fig. 5).

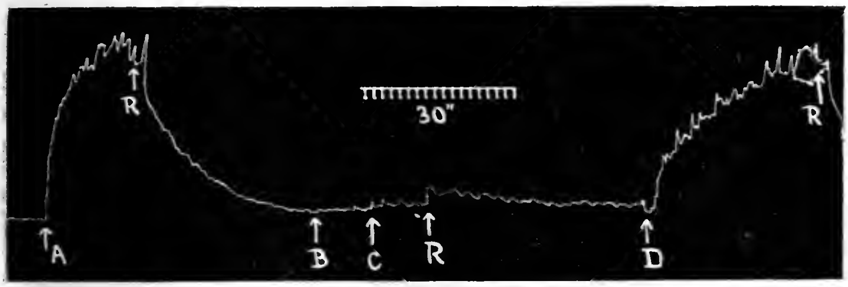

FIG. 5

At *A*, 0.5 cc. butyl alcohol extract solution was added.

At *B*, 0.5 cc. aqueous residue solution was added.

At *C*, 1.0 cc. aqueous residue solution was added.

At *D*, 1.0 cc., obtained by evaporation of 17 cc. of the aqueous residue solution, was added.

R indicates the points at which the Ringer solution was changed. Thus histamine is shown to be practically completely extracted from alkaline solution by butyl alcohol.

2. Histamine hydrochloride. To 0.0057 gram histamine phosphate, dissolved in 30 cc. water, was added very slight excess of colloidal ferric hydroxide solution. A drop of 1 per cent Na_2CO_3 was then added to remove the excess of ferric hydroxide and the solution filtered. After the addition of a drop of 2n. HCl to the clear filtrate it was extracted for twelve hours with butyl alcohol.

The extract and residue were worked up in the usual way, each being dissolved in 40 cc. water and tested on the isolated uterus (fig. 6).

At *A*, 0.5 cc. solution of the butyl alcohol extract was added.

At *B*, 0.25 cc. solution of the aqueous residue was added.

R denotes change of Ringer solution.

Comparisons of the amounts of histamine in extract and residue were made and it was found that the butyl alcohol extract had only one-twelfth the activity of the aqueous residue.

3. Histamine phosphate. A solution of 0.0013 gram histamine phosphate in 20 cc. water was extracted for twelve hours with butyl alcohol. The extract and residue were worked up in the usual way, each dissolved in 20 cc. water and tested on the uterus (fig. 7).

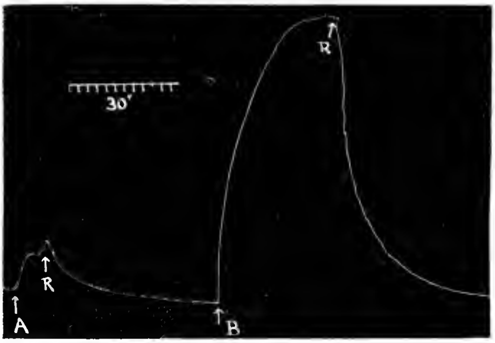

Fig. 6

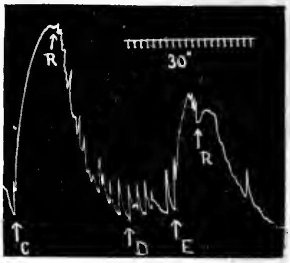

Fig. 7

At *C*, 0.5 cc. solution of the residue was added.

At *D*, 0.5 cc. solution of the extract was added.

At *E*, 2.0 cc. solution of the extract were added.

Quantitative tests were made and it was found that only one-twelfth of the histamine phosphate had been extracted by the butyl alcohol.

A contrast is here to be noted between the rates of extraction of histamine hydrochloride and phosphate on the one hand and of the pituitary uterine stimulant from acid (HCl) solution on the other.

DIFFERENTIATION OF PITUITARY UTERINE STIMULANT AND HISTAMINE

1. Action of alkali

0.0357 gram active pituitary material, extracted by butyl alcohol as described above, was dissolved in 100 cc. water (solution X).

0.002 gram histamine phosphate was dissolved in 30 cc. water (solution Y).

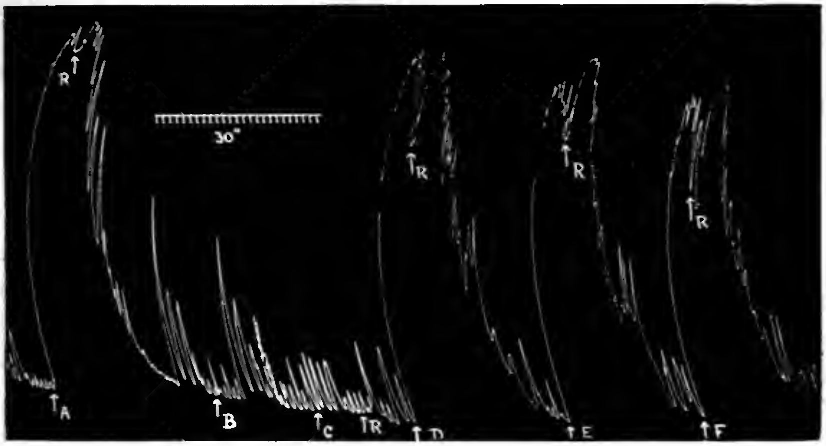

Fig. 8

To 3 cc. solution X were added 3 cc. 2n. NaOH (solution a).

To 3 cc. solution X were added 6 cc. water (solution b).

To 5 cc. solution Y were added 5 cc. 2n. NaOH (solution c).

To 5 cc. solution Y were added 10 cc. water (solution d).

These four solutions then stood at room temperature (17°C.) for two hours. Solutions a and c were then exactly neutralized with 3 cc. and 5 cc. respectively of 2n. HCl, and the whole series tested on the isolated uterus of the guinea-pig (fig. 8).

At *A*, 0.5 cc. solution b was added.

At *B*, 0.5 cc. solution a was added.

At *C*, 2.0 cc. solution a were added.

At *D*, 0.5 cc. solution b was added after 0.02 gram NaCl had been dissolved in it.

This experiment, making the NaCl content equivalent to that of solution a, exhibits a somewhat weaker contraction than that produced at *A*; this is due to the effect of exposure of the uterus to slightly hypertonic conditions.

Just before testing the histamine solutions 0.58 gram NaCl was dissolved in solution d, thus making its NaCl content equal to that of solution c.

At *E*, 0.5 cc. solution c was added.

At *F*, 0.5 cc. solution d was added.

R indicates, as previously, the points at which the Ringer solution was changed.

Thus it was demonstrated that, while the pituitary uterine stimulant is entirely inactivated by alkali, histamine under identical conditions is unaffected.

2. Action of trypsin

To 5 cc. of a 0.1 per cent solution of active pituitary material, obtained by butyl alcohol extraction as described above, 45 cc. 0.1 per cent Na_2CO_3 solution were added. Two portions of 20 cc. of this solution were taken; to one was added a suspension of 0.05 gram Merck's "pancreatin" in 5 cc. water (solution a).

To the other was added a similar suspension of "pancreatin" after it had been boiled for ten minutes (solution b).

A 0.0022 per cent solution of histamine phosphate in 0.1 per cent Na_2CO_3 was prepared.

To 40 cc. of this solution were added 10 cc. of water in which was suspended 0.1 gram Merck's "pancreatin" (solution c).

To a further 40 cc. was added a similar suspension of "pancreatin" after it had been boiled for ten minutes (solution d).

The four solutions were incubated at 37°C. for thirty minutes and then tested on the uterus immediately (fig. 9).

At *A*, 0.5 cc. solution b was added.

At *B*, 0.5 cc. solution a was added.

At *C*, 0.5 cc. solution b was added.

At *D*, 0.5 cc. solution c was added.

At *E*, 0.5 cc. solution d was added.

At *F*, 2.0 cc. solution a were added.

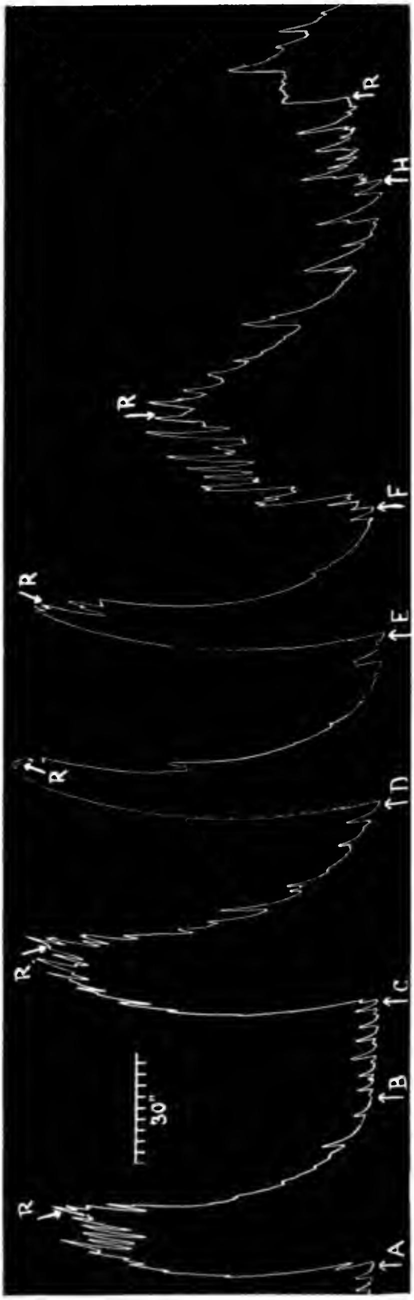

FIG. 9

R indicates the points at which the Ringer solution was changed.

Curve *F* indicated that solution a still possessed, though very slightly, the power of stimulating the uterus. It was thought possible that the changed conditions of the Ringer solution, caused by the introduction of 2 cc. 0.1 per cent sodium carbonate-trypsin mixture, might have something to do with this effect. Accordingly a suspension of 0.1 gram "pancreatin" in 10 cc. water was added to 40 cc. 0.1 per cent Na_2CO_3 and the mixture incubated at 37°C. for thirty minutes. Two cubic centimeters of this solution were then allowed to act on the uterus at *H*. A slight effect was produced, but not so much as in the case of the addition of 2 cc. of solution a. Therefore, while some of the effect in curve *F* may be ascribed to the sodium carbonate and trypsin present, it is obvious that a small amount of the uterine stimulant was still undecomposed.

The contrast with histamine is, however, very marked, for under experimental conditions which almost completely destroy the pituitary uterine stimulant histamine remains entirely unaffected.

3. *Extraction with hot chloroform*

To a solution of 0.005 gram active pituitary material, obtained by butyl alcohol extraction, in 5 cc. water was added sodium carbonate solution until the reaction to litmus was distinctly alkaline.

A solution of 0.0018 gram histamine phosphate in 5 cc. water was similarly treated with sodium carbonate.

Both solutions (in small evaporating dishes) were taken to dryness in a vacuum desiccator over sulphuric acid. After standing overnight each residue was covered with about 10 cc. chloroform and heated on the water bath until the chloroform boiled. The hot chloroform was filtered and each extraction repeated five times.

The chloroform from each filtrate was then distilled off in vacuo. The residues were taken up in water and again distilled

in vacuo to remove traces of chloroform. The final residues were dissolved in water.

The chloroform pituitary extract was made up to 20 cc. The alkaline pituitary residue was dissolved in water, neutralized with HCl, and made up to 20 cc.

The histamine extract and residue were treated similarly and the solutions made up to 20 cc.

These solutions were then tested on the uterus (fig. 10).

Fig. 10

At *A*, 0.5 cc. histamine $CHCl_3$ extract was added.
At *B*, 0.5 cc. histamine residue was added.
At *C*, 0.5 cc. pituitary residue was added.
At *D*, 0.5 cc. pituitary $CHCl_3$ extract was added.
At *E*, 0.2 cc. pituitary residue was added.
At *F*, 2.0 cc. pituitary $CHCl_3$ extract were added.
R indicates change of Ringer solution.

The amounts of histamine in extract and residue were compared quantitatively and it was found that a little over two-thirds of the histamine had been extracted by the chloroform. It is obvious that not more than a trace of the pituitary uterine stimulant passed into solution in the chloroform.

SUMMARY

A method of preparing crystalline residues, very active physiologically, from extracts of the posterior lobe of the pituitary gland is described. It consists in extraction of the dried and

powdered infundibulum with acidulated water, treatment of the solution with colloidal ferric hydroxide and subsequent continuous extraction of the filtrate with butyl alcohol at reduced pressure. This extract yields a crystalline residue which contains all the uterine stimulant, together with some of the pressor principle and contaminating substances.

The uterine stimulant and histamine are not identical, as suggested by Abel and Kubota (1919), but are two distinct chemical substances. The following differences are displayed.

Pituitary uterine stimulant	*Histamine*
Readily extractable from acid (HCl) solution by butyl alcohol at reduced pressure.	Only very slowly extracted from acid (HCl) solution by butyl alcohol at reduced pressure.
Rapidly destroyed by normal sodium hydroxide at room temperature.	Unaffected by normal sodium hydroxide at room temperature.
Rapidly destroyed by trypsin.	Unacted on by trypsin.
Insoluble in boiling chloroform.	Soluble in boiling chloroform.

The only point of similarity observed is that both are readily extracted from alkaline solution by butyl alcohol.

The pituitary uterine stimulant is more readily extracted from acid (HCl) solution than the pressor principle.

REFERENCES

Guggenheim. Biochem. Zeitschr., lxv, 189, 1914.
Aldrich: Amer. Journ. Physiol., xxi, p. xxiii, 1908.
Dakin: Biochem. Journ., xii, 290, 1918.
Engeland and Kutscher: Zeitschr. f. Biologie, lvii, 527, 1912.
Schafer and Vincent: Journ. Physiol., xxv, 87, 1899.
Osborne and Vincent: Journ. Physiol., xxv, 282, 1899.
Abel and Kubota: Journ. Pharm. and Exp. Ther., xiii, p. 243, 1919.
Dale and Laidlaw: Journ. Pharm. and Exp. Ther., iv, 75, 1912.

PERFUSION OF THE MEDULLA OF THE TURTLE WITH ATROPIN, CAFFEIN, AND WITH STRYCHNIN

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The object of this series of experiments was to ascertain if these drugs would produce any registrable action on the cardio-inhibitory centre of the terrapin.

The method used was a modification of that developed by Greene and Peeler (Journ. Pharm., 1915, vii, 591). The turtle was pithed by puncturing the cranium laterally with a sharp nail, care being taken not to injure the medulla. After removal of the plastron, the vagi nerves and carotid arteries were isolated from the cervical tissues; then a normal tracing was taken from the right auricle, and the vagi tested for normal tonicity. Next, the right carotid was connected with the perfusion apparatus, all the structures of the neck except the vagi and carotids were severed, the left carotid was clamped, and the solution run in at a uniform rate for each experiment.

The solution used was frog Locke's. This was fed from a large burette connected with an air pressure system so as to secure adequate pressure for maintaining uniform rate of flow. To this solution was added whatsoever drug was under study. The air in the pressure system entered at the lower end of the burette and bubbled in a fine stream up through the solution; and, while this did not furnish an optimum amount of oxygen to the medulla, probably enough was present in the solution to meet the needs of a terrapin for a brief period.

In this group of experiments three different drugs were used, atropin, caffein, and strychnin. With atropin, 0.02 per cent, and with caffein, 0.04 per cent, no constant change in rate or rhythm of heart action was noted; the general results obtained were

about the same as with the controls where Locke solution without any drug was used. But with strychnin, there occurred a result quite unexpected; this consisted in a diminished force of

TABLE 1

Rate of right auricle; atropin 0.02 per cent

NUMBER	NORMAL	8′	16′	24′	32′	40′	48′	56′	64′	72′	80′	PER MINUTE	SPECIAL CHARACTERISTICS
												cc.	
1	30	27	30	30	30	32	33	30	30	33	32	3	Marked rhythmic tonus wave
2	28	33	30	33	33	33	33	35	30	30	30	1	Increase of tonicity
3	27	34	33	30	33	31	28	28	28	30	30	1	Gradual weakening
4	31	33	37	36	36	37	34	31	27	30	33	1	Gradual weakening
5	45	42	46	45	47	48	45	48	45			1	Slight diminution in tone
6	39	39	43	43	43	42	45	43	42	39		2	Slight diminution in tone
7	36	36	39	42	39	42	39	41	42	42	45	2	Slow diminution in strength
8	32	36	33	34	33	33	30	27	30	28	28	2	Slow diminution in strength
9	30	33	32	32	30	30	30	30	31	30	28	2	Slow diminution in strength
10	39	39	39	39	39	36	36	36	33	34	33	3	Variable tonicity
Average	33	35	36	36	36	36	35	35	34	33	32		

TABLE 2

Rate of right auricle; caffein 0.04 per cent

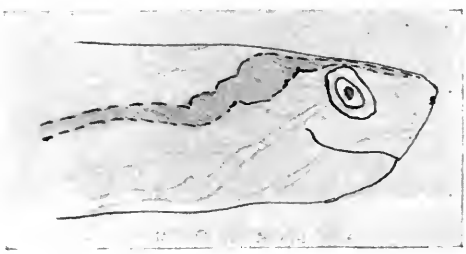
NUMBER	NORMAL	8′	16′	24′	32′	40′	48′	PER MINUTE	SPECIAL CHARACTERISTICS
								cc.	
20	45	42	45	42	45	48	40	4.0	Slow diminution in tonicity
21	40	46	42	41	40	43	40	3.6	Slow diminution in tonicity
22	40	40	42	41	41	41		3.5	No marked change
23	40	42	39	41	40	41	42	4.0	Slight diminution of amplitude
Average	41	42	42	41	41	43	41		
Control	39	39	41	41	42	39		5.0	Slow diminution in tonicity

heart action or a brief temporary cessation, apparently the result of stimulation of the cardio-inhibitory center. The three graphs show the several ways in which complete cessation was manifested.

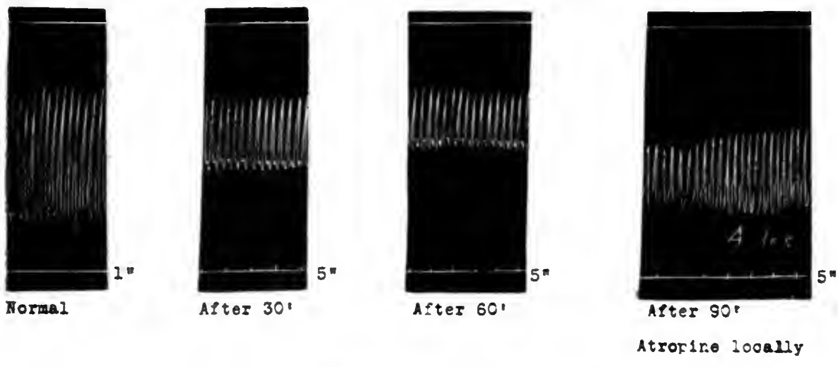
TABLE 3

Rate of right auricle; strychnine sulphate

NUMBER	NORMAL	8′	16′	24′	32′	40′	48′	PER CENT	PER MINUTE FLOW	TIME TO LESS FORCE	TIME TO VAGUS ARREST	TIME TO PART RETURN	TIME TO FULL RETURN
									cc.				
11	39	39	39	39	39	39	31	0.0026	5.0	4′40″			1′
12	38	38	37	37	36	33	33	0.0050	4.0	1′15″		8′	10′
13	42	42	39	39	41	41		0.0050	4.0		1′15″	45″	2′
14	38	48	48	51	51	48	39	0.0050	3.7		2′	5″	2^5′
15	36	40	42	42	45	45	45	0.0033	2.3		10″	5′	55′
16	45	45	45	47	48	47	45	0.0033	3.0	3′45″			2′
17	39	37	36	36	36	38		0.0033	5.0		1′15″	7″	3′
18	39	42	40	40	39	41		0.0033	3.6		1′15″	53″	5′
24	41	40	38	40	39	39		0.0033	5.0		1′	6″	2′
25	45	45	43	45	46	43		0.0033	4.4		2′	50″	5′
Average	40	41	41	41	42	41	38		4.0		1′16″		



HEAD OF STRIPED TURTLE, SHOWING RELATIVE POSITION OF MEDULLA

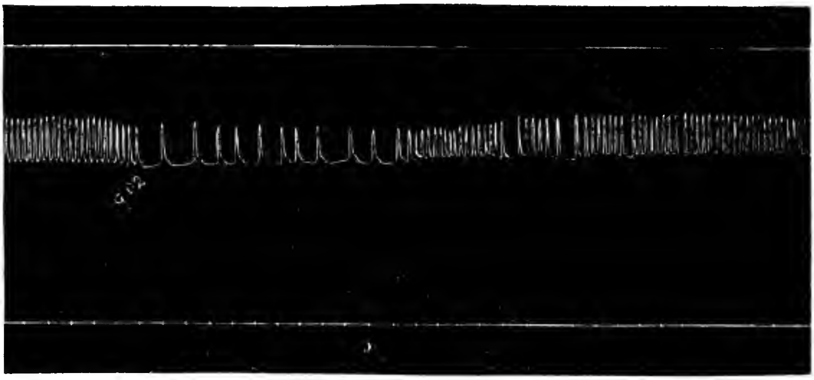


EXPERIMENT NO. X. ATROPIN

5"

Graph from Experiment 17

Showing vagus inhibition occurring one and a quarter minutes after beginning of perfusion of 0.0033 per cent strychnine.



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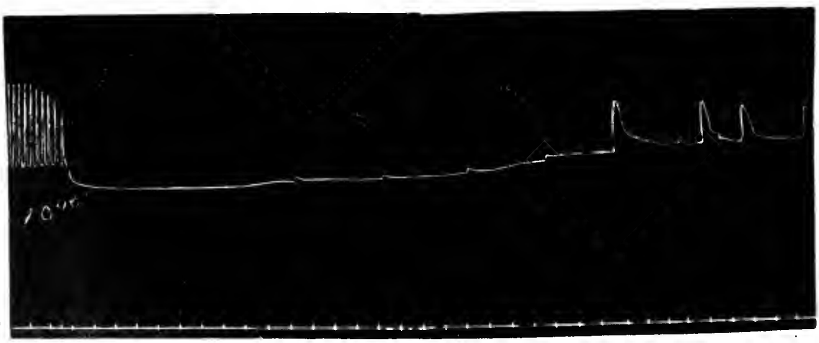
Graph from Experiment 24

Showing vagus inhibition occurring one minute after beginning perfusion of 0.0033 per cent strychnine.



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Graph from Experiment 25

Showing vagus inhibition occurring two minutes after beginning of perfusion of 0.0033 per cent strychnine.

SUMMARY

These few experiments seem to indicate that on the isolated medulla of the striped turtle, atropin, 0.02 per cent, and caffein, 0.04 per cent, exert little or no registrable influence; whereas strychnin, 0.0033 per cent, produces a prompt, though temporary, stimulation of the cardio-inhibitory center.

COMPARATIVE TOXICITY OF LOCAL ANESTHETICS AND OF ANTIPYRETICS FOR EARTHWORMS

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Introduction. The use of earthworms for the bio-assay of anthelmintics was discussed in a previous paper (1). It was pointed out that this method of bio-assay is convenient and furnishes information of practical value; but that judgment is necessary in transferring the results to clinical problems.

It appeared interesting to determine whether these animals would likely be useful for bio-assays of other groups of drugs. In principle, any life-test can measure differences of concentration between two samples of the same chemical substances, with more or less accuracy; but when it comes to comparing different chemical substances, the value of the results depends on the identity of the response.

For instance, certain substances may kill earthworms by paralysis of the smooth muscle; they may kill mammals by paralysis of the respiratory center. The mechanism of the toxicity in the two cases has practically nothing in common; and consequently the relative toxicity for earthworms would throw no light on the relative toxicity for mammals.

The antipyretics and the local anesthetics produce death in mammals by paralysis of the heart or of the respiratory center. In earthworms, the mechanism of death must be different. It would therefore not be expected that the toxicity would be parallel.

Methods. In the following experiments, the toxicity for earthworms was determined by methods described in the previous paper (1). The anesthetics were used in the form of

hydrochlorids; the insoluble anesthetics (anesthesin, etc.) being dissolved by the addition of a minimum of dilute hydrochloric acid; any excess being neutralized by sodium bicarbonate. Phenylcinchoninic acid was dissolved in 0.1 per cent sodium bicarbonate. The tests on tadpoles were performed by placing 3 tadpoles, length of body 4 mm., exclusive of tail into 100 cc. of the solution. The effects as described refer to twenty-four hours for earthworms and two hours for tadpoles. The experiments were carried out during the summer of 1919 by Miss J. R. Collacott.

TOXIC CONCENTRATIONS OF LOCAL ANESTHETICS FOR EARTHWORMS

Table 1 gives the concentration in which the worms died or survived for one day.

TABLE 1

Toxic concentrations of local anesthetics for earthworms. The drugs are arranged in descending order of toxicity

	CONCENTRATIONS	
	Fatal	Survived
Cycloform	1:50,000	1:75,000
Apothesin, Holocain	1:25,000	1:50,000
Anesthesin, Cocain, Orthoform—New, Procain, Propaesin	1:10,000	1:25,000
Nirvanin	1:7,500	1:10,000
Beta-eucain	1:5,000	1:7,500
Alypin	1:1,000	1:10,000

The questions of the applicability of these data to mammalian toxicity can be approached by comparing the order of the drugs in this table with the minimal fatal dose for cats, with intravenous injection. Eggleston and Hatcher's (2) data for this are shown in table 2.

TABLE 2

Minimal intravenous fatal dose of local anesthetics for cats (Eggleston and Hatcher). The dosage is in milligrams drug per kilogram of body weight

Alypin, holocain	10.0
Beta-eucain	12.5
Cocain	15.0
Apothesin	20.0
Tropacocain	20–25
Stovain	25–30
Nirvanin	30–35
Procain	40–45

Table 3 gives the order of toxicity of the drugs that appear in both tables. There is a fair agreement for holocain, cocain, procain and nirvanin, but disagreement for apothesin, beta-eucain and alypin. These disagreements are so serious that they would preclude earthworms as suitable test objects for the clinical toxicity of local anesthetics.

TABLE 3

Comparison of the order of toxicity of local anesthetics for earthworms and for cats

	EARTHWORMS	CATS
Apothesin	1	5
Holocain	2	2
Cocain	3	4
Procain	4	6
Nirvanin	5	7
Beta-eucain	6	3
Alypin	7	1

TOXICITY OF LOCAL ANESTHETICS FOR TADPOLES

The following were tried in concentrations of 1: 10,000 and 1: 100,000:

Anesthesin, apothesin, cocain, cycloform, orthoform-new, procain and propaesin.

In each case, the higher concentration killed, and the lower was survived for twenty-four hours.

Alypin, holocain and nirvanin also killed in 1: 10,000.

It is possible that the data on tadpoles would conform much better to those on mammals; but the season was too far advanced to make further experiments.

TOXICITY OF ANTIPYRETICS FOR EARTHWORMS

This is shown in Table IV. The observation that phenacetin is ten times more toxic than acetanilid suffices to show that earthworms also fail as test objects of the clinical toxicity of antipyretics.

TABLE 4

Toxic concentrations of antipyretics for earthworms

	CONCENTRATIONS	
	Fatal	Survived
Phenacetin	1:50,000	1:75,000
Salicylic acid	10,000	25,000
Cinchophen	10,000	25,000
Acetanilid Acetyl salicylic acid	5,000	7,500
Antipyrin Sodium salicylate	1,000	2,500
Pyramidon	500	1,000
Melubrin	100	500

TOXICITY OF ANTIPYRETICS FOR TADPOLES

These survived twenty-four hours in 1:10,000 solutions of acetanilid, antipyrin and sodium salicylate. They died in 1:1000 solutions of acetanilid and antipyrin.

CONCLUSIONS

The investigation furnishes data of the toxic concentrations of local anesthetics and of antipyretics for earthworms.

These are not parallel to the toxicity for mammals, since the mechanism of the fatal effects is different.

BIBLIOGRAPHY

(1) SOLLMANN: J. Pharm. and Exper. Therap., 1918, xii, 129.
(2) EGGLESTON AND HATCHER: J. Pharm. and Exper. Therap., 1919, xiii, 444.

ON THE ANTHELMINTIC ACTION OF BENZYL ALCOHOL AND BENZYL ESTERS

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The present author has already called attention elsewhere to the interesting properties of the benzyl esters, benzyl benzoate and benzyl acetate, in relation to the gastro-intestinal tract (1). He has also shown that the newly discovered local anaesthetic, benzyl alcohol, possesses similar properties in relation to smooth muscle organs (2). Furthermore, the author has shown in conjunction with V. E. Nelson that benzyl alcohol possesses distinct antiseptic properties (3); and in conjunction with Homer Fisher pointed out that the benzyl esters are toxic for certain protozoa (4). It was therefore interesting to inquire whether the above drugs are also toxic for worms, and to this end, the present investigation was undertaken.

METHOD

Sollman in his valuable contribution on Anthelmintics published in this Journal (5), showed that "all clinical anthelmintics are markedly toxic to earth worms," and that "this simple test may therefore be used for determining whether a given substance has any anthelmintic properties." His method was employed in testing the benzyl compounds in the present research. Fresh earthworms were placed in conical urine glasses each containing 100 cc. of water on the one hand, and 100 cc. of the solution tested on the other, and the effect of the drugs was studied over several hours at a time. In case of benzyl alcohol solutions of the drug were employed. In case of the benzyl esters, benzyl benzoate and benzyl acetate, the substances being very slightly soluble in

water, emulsions or suspensions of the same had to be used. In addition to the earth worms (*lumbricus terrestris*) observations were also made with the same drugs on the behavior of the large round worms (*ascaridia*) found so commonly in the intestines of freshly slaughtered pigs.

RESULTS

Experiments were made on both earth worms and round worms of the pig with benzyl alcohol, benzaldehyde, benzyl acetate and benzyl benzoate. It was found that all of these drugs exerted a toxic effect on the worms but not in the same degree. The least effective was benzyl benzoate. Its weak action, however, must be for the most part due to its poor solubility and penetrating power. Benzyl alcohol was found to be the most powerful anthelmintic of the drugs studied. A 0.5 per cent solution of it, and even weaker solutions killed earthworms rapidly. Benzaldehyde came next in its efficiency and benzyl acetate was third.

The following protocols will illustrate some of the findings:

I. Experiment October 2, 1918

A. Three earthworms placed in 100 cc. of tap water. Added ethyl alcohol sufficient to make a 1 per cent solution. Worms are at first slightly excited but soon return to normal condition. Alive next day.

B. Three earthworms placed in 100 cc. of tap water, water poured off and replaced by 100 cc. solution of benzyl alcohol 1 per cent. Primary stimulation of the worms, followed by an anaesthetic and depressant effect. Five minutes later the worms are dead and cannot be revived.

II. Experiment October 2, 1918

A. Ethyl alcohol 1 per cent solution has practically no effect on earth worms. Worms are found alive next morning.

B. Solution of 0.5 per cent of benzyl alcohol first stimulates the worms and then kills them in ten minutes.

III. Experiment October 3, 1918

A. Earth worms placed in tap water are lively and well while under observation for several hours.

B. A 1 per cent emulsion of benzyl acetate first excites the parasites and then kills them in twenty minutes.

IV. Experiment October 3, 1918

A. Earth worms placed in ordinary tap water are alive for several hours while under observation.

B. A 1 per cent solution of benzyl benzoate produces little effect after half an hour and slight depression after two hours. On the following day, the control worms were still alive but those treated with benzyl benzoate were dead.

V. Experiment October 5, 1918

A. Large round worms from the intestines of a freshly slaughtered pig are placed in warm saline solution, in two conical glasses.

A. Ethyl alcohol 2 per cent slightly excites or stimulates the movements of the worms but does not kill them. The worms are found alive next day.

B. A 0.5 per cent solution of benzyl alcohol produces primary stimulation of the movements, followed by paralysis and death after about one hour.

DISCUSSION

It will be noted that benzyl alcohol is quite toxic for worms. The author has also found a similar action though not as rapid on using solutions of benzaldehyde. The more rapid onset of death in case of the earthworm as compared with the roundworm of the pig is undoubtedly due to the quicker penetration of the drug in the former, and the poorer penetration through the thick chitinous walls of the latter. It may be added that the author noted in case of earthworms that the parasites underwent decomposition after death in solutions of benzyl alcohol much more slowly than when they were killed with strong solutions of ethyl alcohol, say 5 per cent. This is undoubtedly due to the greater antiseptic power of benzyl alcohol.

The anthelmintic action of benzyl alcohol and benzaldehyde naturally raises the question as to the advisability of their administration in clinical cases. Both benzyl alcohol and benzaldehyde being but little toxic when taken by mouth could be safely administered for that purpose. The question as to their usefulness and suitability for clinical use therefore depends entirely on whether their anthelmintic action can be brought to bear in sufficient concentration on parasites in the intestinal canal on the one hand, and whether these drugs are equally toxic to the parasites found in the human being as they are for the parasites studied above, on the other. The author had administered benzyl alcohol and benzaldehyde in enteric capsules to a few patients suffering from tapeworms and has obtained an anthelmintic effect, though the action of the drugs in the few cases to which it was administered was not a completely efficacious one. The results, however, are interesting from the scientific point of view. Whether the drugs promise to be of real value in therapeutics can only be determined by an extensive clinical study which is at present lacking.

REFERENCES

(1) Macht: Jour. Pharmacol. and Exper. Therap., 1918, xi, 419.
(2) Macht: Jour. Pharmacol. and Exper. Therap., 1918, xi, 263.
(3) Macht and Nelson: Proc. Soc. Exper. Biol. and Med., 1918, xvi, 17 (1392).
(4) Macht and Fisher: Jour. Pharmacol. and Exper. Therap., 1917, x, 95.
(5) Sollmann: Jour. Pharmacol. and Exper. Therap., 1918, xii, p. 129.

THE ACTION OF ADRENALIN ON THE HEART

II. THE MODIFICATION OF THE ACTION OF ADRENALIN BY MORPHIN

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Oliver, Schäfer (1) and others, while investigating the action of adrenalin used morphin with ether or chloroform as an anesthetic. According to Brown (2), Cybulski (3) and as I have recently found in my work on turtles (4), adrenalin exerts a direct action on the medullary centers while morphin in the dog produces a slow irregular pulse from powerful stimulation of the vagus centers (Cushny (5)). Many investigators, who use morphin-ether anesthesia are able to elicit marked inhibition of the heart, following an injection of adrenalin. This inhibition has been attributed to stimulation of the cardio-inhibitory center by the rise in blood-pressure (6).

While studying the action of adrenalin on the dog's heart, it occurred to me that morphin by its central action might influence the effect of adrenalin. In the experiments recorded below, especial attention has been given to the modification of the adrenalin action by this alkaloid.

The animals were prepared in the usual manner for recording carotid pressure. The drugs were given by intravenous injection into the femoral vein and were washed in with 5 cc. of normal salt solution from a burette. After normal tracings were recorded, adrenalin in varying amounts was injected; and when the blood pressure had returned to normal 0.008 gram of morphin sulphate was given intravenously. The results of a subsequent dose of adrenalin were then compared with those produced previously to the morphin. The blood

pressure in some of the experiments was raised artificially either by stimulation of the splanchnic nerve, or preferably by occluding the abdominal aorta. After this the vagi were generally cut, and adrenalin again injected. The respiration was not always recorded, but when recorded mention is made of it.

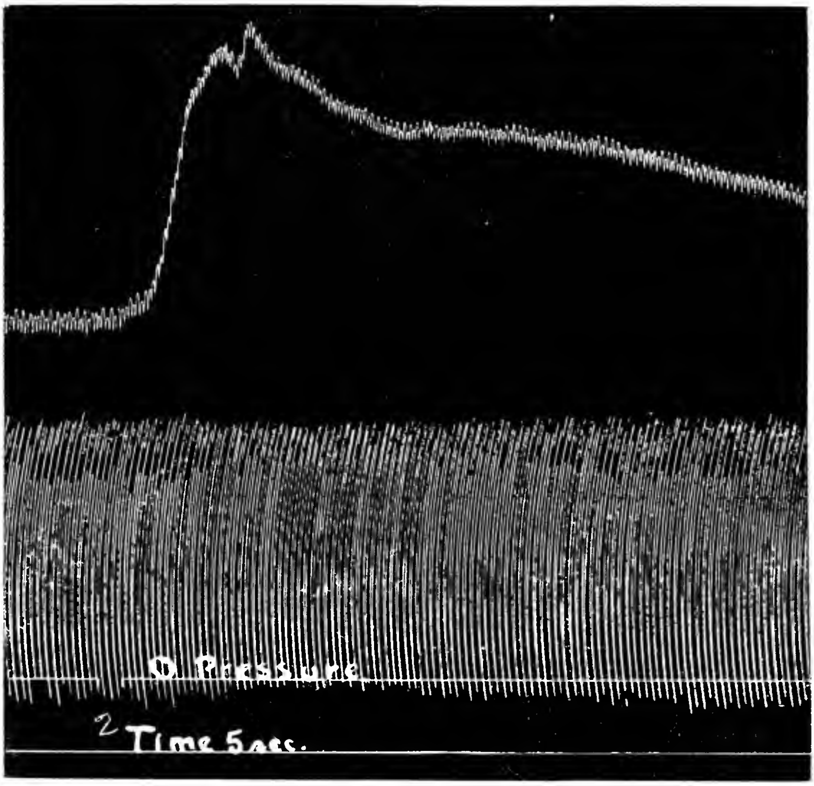

Fig. 1. Rise in Pressure Produced by the Intravenous Injection of 1 cc. 1–2,000 Adrenalin Previous to Morphin

Note that only acceleration was effected

In a series of seventeen dogs inhibition was produced in fourteen or eighty-two per cent. In two young animals and one old pregnant bitch slowing was not elicited by any amount of adrenalin either before or after morphin. It is probable that the vagus mechanism is not as sensitive or as well developed

in the young dog as in the old. I found five dogs in this series in which inhibition was not produced previous to but did occur after morphin.

Although unable to make such statements as have Meek and Eyster (7), who state that intravenous injections of physiological amounts of adrenalin in dogs with good vagal tone invariably causes a decrease in heart rate, or to conclude as does Hoskins and Lovelette (8), who used dogs with rapid pulse and high blood pressure, that adrenalin produces not only an increase in blood pressure but generally also an accelerated pulse, I have found that as a rule dogs with high blood pressure and quickened pulse (conditions which indicate poor vagal tone) adrenalin alone produces acceleration only, and inhibition only after morphin had been given.

Stewart and Rogoff (9) in a recent article state the concentration of adrenalin in the serum which they think is very nearly the possible normal maximum to be 1:560,000. I have found this physiological dose quite ineffective. In fact in only one dog under chloroform anesthesia was inhibition elicited with 1 cc. of 1–100,000 adrenalin in any of our experiments. I shall report the effect of chloroform on adrenalin action in a subsequent paper. In the present work we used large doses, but generally employed a 1–10,000 solution in amounts within therapeutic limits; while doses of 1 cc. of 1–2000 adrenalin have been injected without any inhibition being produced.

Stewart and Rogoff (10) have found that a fresh solution of adrenalin hydrochloride yielded on colorimetric examination 56 to 80 per cent of base. I am of the opinion that the powder is more stable but find that rapid oxidation takes place even in dark stoppered bottles. I used the tablet adrenalin of Parke, Davis and Company, fresh solutions being prepared before injection.

Experiment I. This experiment is one of a series of eleven (11) which agree in all but a few minor details. They were performed in order to determine the influence of morphin on adrenalin action.

The normal pulse was 171; systolic pressure 126 mm. Hg and respiration 108. Following the injection of 1 cc. of 1–10,000 adrenalin

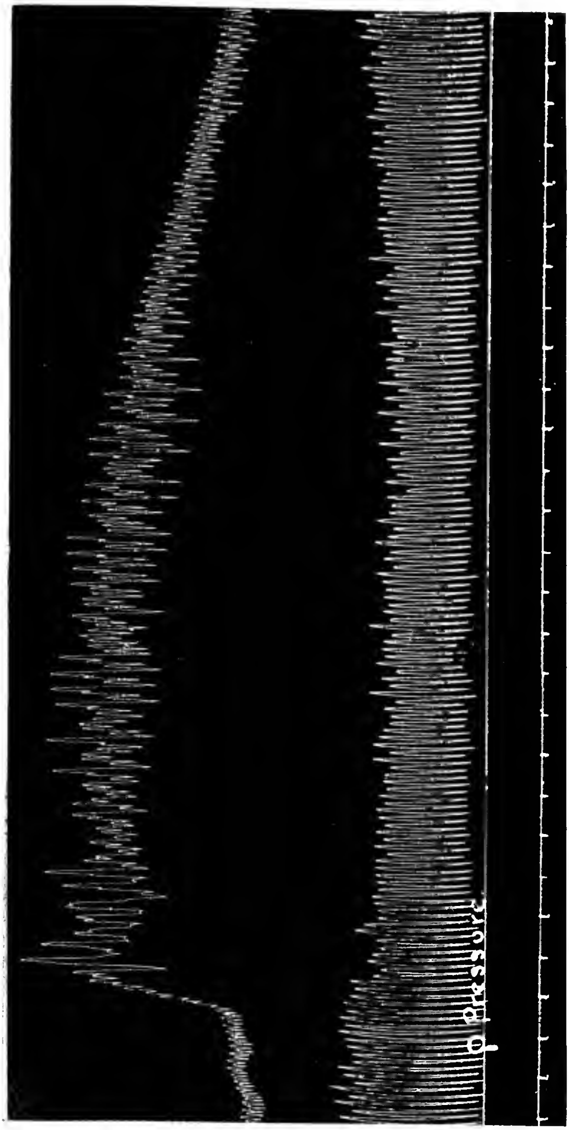

Fig. 2. Rise in Pressure Produced by the Intravenous Injection of Adrenalin 1 cc. 1–2,000 After 0.016 Gram of Morphin

Note the pronounced inhibition and the decrease in amplitude of respiration

the pressure rose to 183 mm. Hg and the heart rate became 189, an acceleration of 10.6 per cent. An injection of 1 cc. of 1–2,000 adrenalin increased the pressure 72.7 per cent and the pulse 27 per cent. 0.016 gram of morphin given intravenously did nor affect the heart rate although the respiration slowed a little. 1 cc. of 1–10,000 adrenalin now produced a rise of 57.6 per cent and an acceleration of 2 per cent. It is probable that not sufficient time was given for the morphin to exert

TABLE 1

Pulse	Pressure: Systolic	Pressure: Diastolic	Respiration	Rise in pressure	Inhibition	Remarks
				per cent	per cent	
171	126	120	108	45.2		Normal
189	183	178	108		−10.6	After 1 cc. 1–10,000 adrenalin
168	124	114	99			Before 1 cc. 1–2,000 adrenalin
213	214	208	102	72.7	−27.0	After 1 cc. 1–2,000 adrenalin
162	112	106	111			Previous to morphin
162	112	106	102			Before 1 cc. 1–10,000 adrenalin and after morphin
165	176	170	96	57.6	−2.0	After 1 cc. 1–10,000 adrenalin
105	204	192	74	82.1	36.0	After 1 cc. 1–2,000 adrenalin
141	100	92	72			Before 1 cc. 1–10,000 adrenalin
129	154	142	69	54.0	9.0	After 1 cc. 1–10,000 adrenalin
138	112	102	69			Before 1 cc. 1–2,000 adrenalin.
90	178	164	69	59.0	34.8	After 1 cc. 1–2,000 adrenalin
150	98	90	87			Before 1 cc. 1–10,000 adrenalin
135	152	142	78	55.1	10.0	After 1 cc. 1–10,000 adrenalin
131	110	100	71			Before 1 cc. 1–2,000 adrenalin
74	194	142	68	76.5	43.5	After 1 cc. 1–2,000 adrenalin
138	86	80	54			Before physostigmin
147	80	76	54	−7.0	−6.5	After physostigmin and before 1 cc. 1–2000 adrenalin
63	20	113	Inhibition	50.0	57.0	After 1 cc. 1–2000 adrenalin

its action for subsequent doses of adrenalin produced inhibition. 1 cc. of 1–2,000 adrenalin slowed the heart 36 per cent while increasing the pressure 82.2 per cent. 1 cc. of 1–10,000 adrenalin effected 9 per cent inhibition with a rise of 54 per cent. After physostigmin the heart accelerated 6.5 per cent. 1 cc. of 1–2000 adrenalin now produced a slowing of 57 per cent with a rise of 50 per cent. From this experiment it is quite evident that the slowing is in proportion to the dose and not the

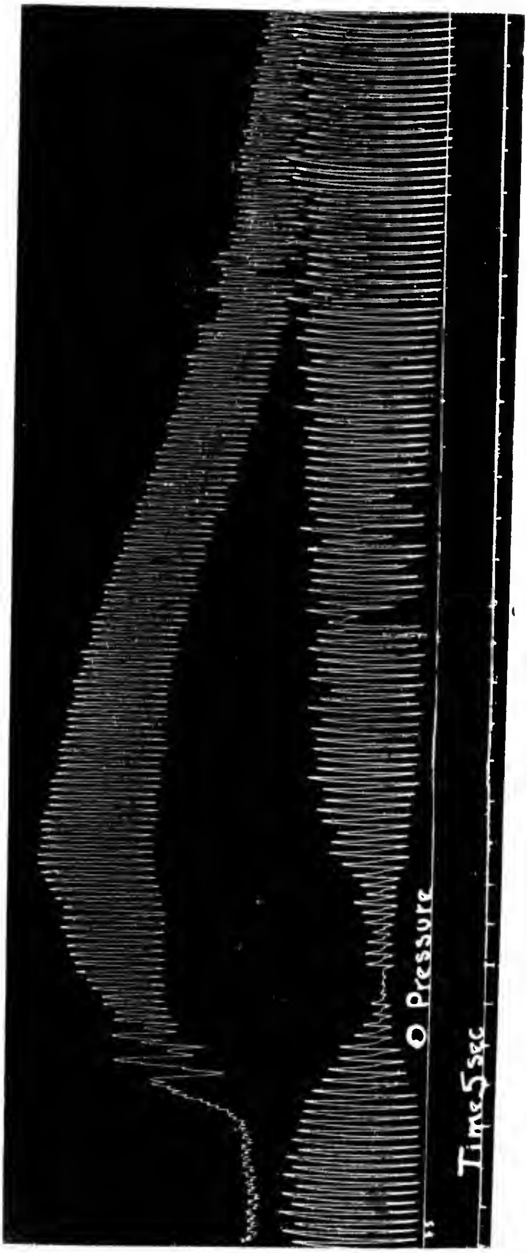

FIG. 3. RISE IN PRESSURE PRODUCED BY THE INTRAVENOUS INJECTION OF ADRENALIN 1 CC. 1–2,000 AFTER MORPHIN AND AFTER PHYSOSTIGMIN HAD BEEN GIVEN TO SENSITIZE THE VAGUS ENDINGS

Note the marked inhibition of the heart and respiration

rise in pressure. The marked results after physostigmin are probably due to the sensitizing effect of the drug on the vagus endings. This action of adrenalin is central for after section of the vagi inhibition is not effected. The entire experiment is given in table 1.

Experiment 2. A series of four experiments all of which agreed in essentials and of which this one is typical were performed to determine (1) Whether morphin sensitized the vagus center, and (2) whether the slowing produced by adrenalin after morphin was greater than that produced by artificial raising the pressure.

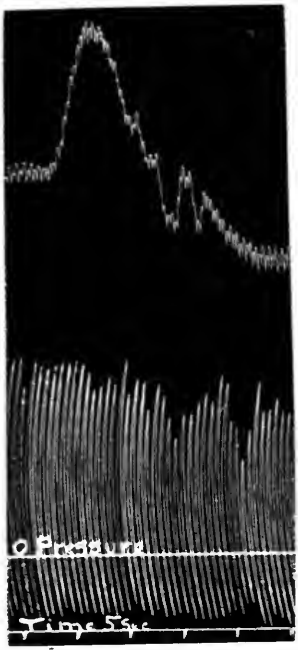

FIG. 4. EFFECT OF 1 CC. OF 1–10,000 ADRENALIN PREVIOUS TO MORPHIN

A male dog, weighing about 8 kilos; fairly well nourished. The normal pressure was 130 mm. Hg and the pulse rate was 168. 1 cc. of 1–10,000 adrenalin produced a rise in pressure followed by a fall during which the pressure became subnormal (see fig. 4). During the fall the pulse increased to 210. Pressure was increased by partially occluding the abdominal aorta, for stimulation of the splanchnics did not produce the required rise. The pressure was increased from 96 mm. to 160 mm. Hg. No slowing occurred (see Fig. 5). 0.008 gram of morphin

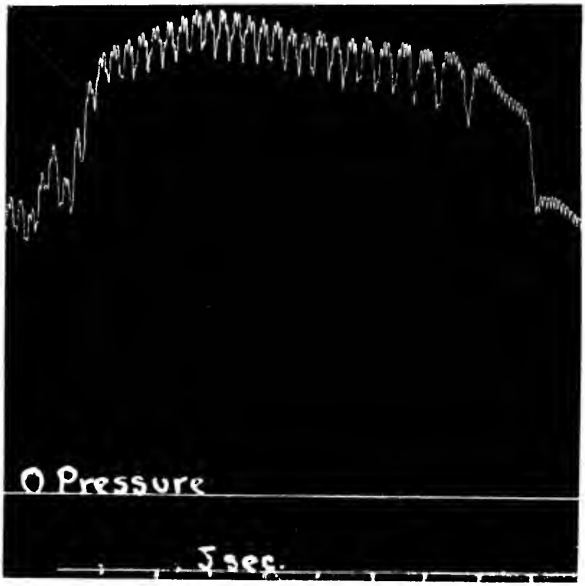

FIG. 5. EFFECT OF INCREASING THE PRESSURE BY OCCLUSION OF THE ABDOMINAL AORTA PREVIOUS TO THE USE OF MORPHIN

Note that no inhibition has occurred

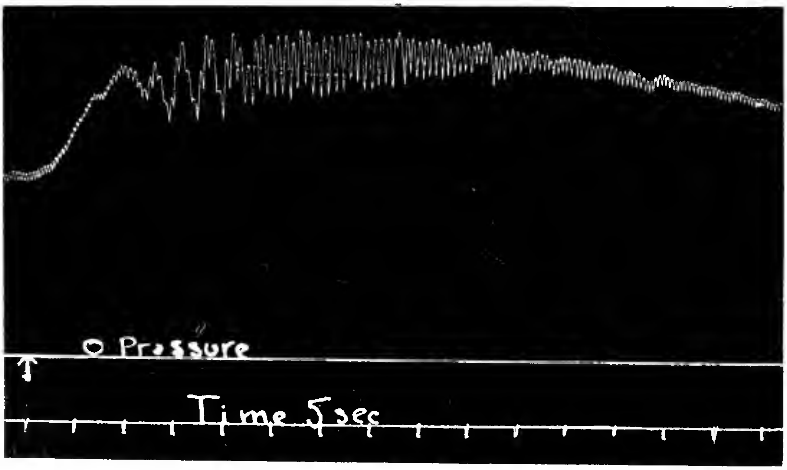

FIG. 6. EFFECT OF 1 CC. 1–10,000 ADRENALIN AFTER MORPHIN HAD BEEN ADMINISTERED

sulphate was injected. The pressure was again mechanically increased from 46 mm. to 116 mm. Hg. There now occurred distinct evidence of

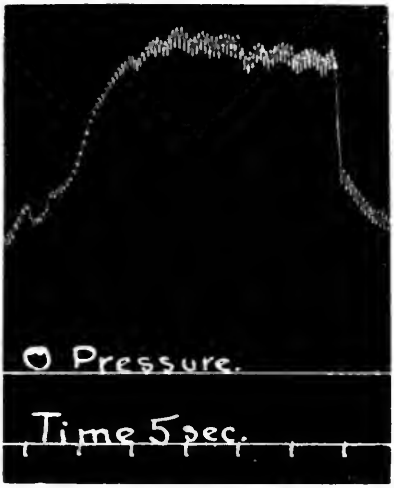

FIG. 7. EFFECT OF OCCLUDING THE ABDOMINAL AORTA AFTER MORPHIN HAD BEEN GIVEN

Compare with figure 5

TABLE 2

PRESSURE			RISE IN PRESSURE	INHIBITION	REMARKS
Pulse	Systolic	Diastolic			
			per cent	*per cent*	
168	130	126			Normal
210	176	166	35.4	−25.0	After 1 cc. 1–10,000 adrenalin
180	96	92			Before occlusion of abdominal aorta
180	160	157	66.7	0	After occlusion of abdominal aorta
192	46	43			Before occlusion of abdominal aorta after morphin
150	116	106	152.2	21.8	After occlusion of abdominal aorta after morphin
222	64	60			Before 1 cc. 1–10,000 adrenalin
84	104	100	62.3	62.1	After 1 cc. 1–10,000 adrenalin
216	44	41			Before occlusion of abdominal aorta
162	114	108	159.0	25.0	After occlusion of abdominal aorta

vagus inhibition the pulse slowing from 192 to 150. 1 cc. of 1–10,000 adrenalin produced a rise in pressure from 64 to 104 and then dropped to

94. Distinct partial inhibition was effected the pulse dropping from 222 to 84 (see fig. 6). Pressure was again increased artificially from 44 mm. to 114 mm. Hg. Again evidence of vagus stimulation was effected the heart slowing from 216 to 162 (see fig. 7). It should be

TABLE 3

Pressure			Rise in pressure	Respiration	Inhibition	Remarks
Pulse	Systolic	Diastolic				
			per cent		*per cent*	
132	110	92		54		Normal
132	110	92		—		At beginning of asphyxia
130	90	75	−18.1	—	1.5	At end of asphyxia
124	160	130		40		After asphyxia
124	134	114		48		Before 1 cc. 1–20,000 adrenalin
116	150	122	10.6	52	6.4	After 1 cc. 1–20,000 adrenalin
124	116	100				At beginning of asphyxia
88	70	58	−39.7		29.0	At end of asphyxia
108	119	100		48		Before 1 cc. 1–20,000 adrenalin
106	152	140	20.6	54	1.9	After 1 cc. 1–20,000 adrenalin
106	105	74		64		Before 1 cc. 1–10,000 adrenalin
112	160	114	34.3	64	−5.7	After 1 cc. 1–10,000 adrenalin
102	85	58		46		Before 1 cc. 1–2,000 adrenalin
72	190	140	55.2	50	49.0	After 1 cc. 1–2,000 adrenalin
108	86	72		50		Before 1 cc. 1–10,000 adrenalin
110	136	114	58.1	48	−1.9	After 1 cc. 1–10,000 adrenalin
98	76	52		42	3.6	After 0.008 grm. morphin
90	104	66	36.8+	Irreg.	8.2	After 1 cc. 1–20,000 adrenalin
92	90	54		40		Before 1 cc. 1–20,000 adrenalin
84	124	76	37.7	40	8.7	After 1 cc. 1–20,000 adrenalin
76	134	88	48.8	32	17.4	After 3rd cc. 1–20,000 adrenalin
120	96	78	—			Before 1 cc. 1–20,000 adrenalin
56	174	124	81.3	8	53.3	After 1 cc. 1–20,000 adrenalin
82	130	80				At beginning of asphyxia
134	102	98	−21.6		−64.6	At end of asphyxia
69	126	120		24		Before 2 cc. 1–20,000 adrenalin
38	150	137	19.0+	24	45.0	After 2 cc. 1-20,000 adrenalin
100	116	100		40		Before 1 cc. 1–20,000 adrenalin
54	136	130	17.2+	40	46.0	After 1 cc. 1–20,000 adrenalin
120	16			30		Before 0.4 cc. 1–20,000 adrenalin
68	124			30	43.3	After 0.4 cc. 1–2,000 adrenalin

noted in this experiment that while occlusion of the aorta increased the pressure 159 per cent, the inhibition was only 25 per cent, and that adrenalin causing a rise in pressure of 62.3 per cent produced a slowing of 62.1 per cent (table 2).

The experiment included below is one of two experiments agreeing in detail performed to determine: (1) Whether asphyxia effects inhibition of the heart and (2) Whether morphin has any influence on this action. It will be noticed that after the morphin asphyxia produced acceleration while previous to it inhibition was effected. The inhibition is in proportion to the dose of adrenalin rather than to the rise in pressure. Previous to the morphin 1 cc. of 1–20,000 adrenalin produced an inhibition of 6.4 per cent while after it the slowing was 46 per cent. The asphyxia lasted about four minutes. The experiment is given in table 3.

DISCUSSION

It is the common opinion that inhibition of the heart following adrenalin is due "to the high blood pressure which induces congestion of the brain and arouses the vagus center to activity" (6). After section of the vagi this inhibition is not observed. Morphin exerts a central stimulating action on the vagus center producing a slow irregular pulse. In small amounts as I have used there is generally no decided vagus action although the respiratory center is readily acted upon. Adrenalin as I have reported and as other investigators concluded, has a direct action on the medulla. The increased inhibition produced by adrenalin following the use of morphin may be due to either of two factors (1) sensitization of the medullary center by the morphin or (2) synergistic or additive action of adrenalin and morphin.

Jackson and Ewing (11) found that morphin increases the reflex excitability of the vagus centers. Morphin also excites the cord (12) while cerebral depression is primary (13). In my work the amount of morphin given was just enough to slightly depress the respiratory center. It is probable that if morphin did exert a sensitizing effect, under the conditions of our experiments the action would be manifest. Using a different method than did Jackson and Ewing to determine the increased reflex excitability of the vagus center, I have found (experiments VII, VIII, IX, X), that morphin, while slightly depressing the respiratory center which seems to be much more sensitive to morphin than the other medullary centers, sensitizes the vagus

centers. Occlusion of the abdominal aorta above the mesenteric branches, and stimulation of the splanchnics simulate the stimulating effect of the sympathetic myoneural junctions in the splanchnic area by adrenalin and hence we may look for similar results to be produced by adrenalin and occlusion of the aorta. But the facts are as shown by these experiments, the inhibition produced by adrenalin is greater than that produced by increasing the pressure artificially; and that the inhibition effected by artificially raising the pressure is greater after morphin than previous to it. It is evident then that morphin does increase the excitability of the vagus center as Jackson and Ewing found.

It may seem probable to some that since the respiratory and cardio-inhibitory centers are in proximity that they should be acted upon similarly by the same drugs. But the fact is that morphin while depressing the respiratory center actually sensitizes the vagus center. That this alkaloid acts differently upon these medullary centers is no more startling than the fact that atropine selectively acts upon the autonomic endings, and not on the sympathetic; or that morphin while chiefly depressant in the dog induces wild excitement in the cat; or that aconite affects only the sensory nerve endings; or that curare acts on the nerve endings to striated muscle. The result of our experiment I think shows a difference in action of morphin on the medullary centers.

Since morphin sensitizes the centers one might suppose that they would respond more quickly and strongly to a rise in blood pressure and hence produce an aggravated slowing. However I think that experiments VII, VIII, IX, and X amply prove that it is not the rise in pressure that is the cause of the slowing. In one experiment adrenalin produced a greater slowing, 62.1 per cent, after a smaller rise in pressure 62.3 per cent, than was effected by a greater mechanical rise of 159 per cent. I have found in experiments of another series that 1 cc. of 1-10,000 adrenalin might produce a greater rise in pressure than 1 cc. of 1-2000 adrenalin, but that the latter dose produced very marked slowing where as only acceleration was produced by the former dose. I have also found that the inhibition is in

relation to the size of the dose rather than in relation to the rise of pressure as I would expect were the latter the causative agent.

The second possible cause for this aggravated adrenalin inhibition following the use of morphin is either an adrenalin-morphin additive or synergistic reaction. I feel that it is not an additive action from the fact that the quantities of morphin I used were not sufficient to produce any signs of vagus inhibition. Therefore, I am inclined to believe that adrenalin and morphin have a synergistic central action.

The rise in blood pressure probably plays little part in the slowing of the heart rate for the following reasons: (1) The degree of inhibition is not proportional to the rise in pressure as we should expect, but is in direct proportion to the size of the dose. (2) The centers being very delicate in reaction if stimulated by high pressure would not continue to respond when the pressure returned to normal; but I have shown slowing persists after return to the normal pressure. (3) It is hardly permissible to assume without more adequate basis, and from my experiments I am unable to offer any, that the cardio-inhibitory center is set to react to a definite rise in pressure and is not influenced by one lesser or greater than this specific one. However, in none of my experiments have I found that the rise in pressure has a definite relation to the degree of a slowing. (4) If blood pressure alone were the cause I would expect an equal degree of slowing previous to and after morphin to be produced by an equal rise in pressure. This is amply disproved in my experiments.

A third possibility is that adrenalin sensitizes the centers and that morphin is then able to produce inhibition by its stimulating powers. I feel that this is not the cause however, since sufficiently large doses which cause an extreme rise in pressure have been given previously to morphin without any inhibition being produced. If the increased blood pressure stimulated the vagus centers, and I think it has but little action; and if adrenalin sensitized the vagus center I should expect slowing to occur in the cases mentioned. However, I

am unable to draw any further disproof of this postulation from my experiments.

Starling (14) in writing of the cardio-inhibitory center states that "anything which interferes with the gaseous exchange of the center calls forth an increased state of activity of the center." "This is due to one of two things (1) excess of carbon dioxide or (2) lack of oxygen. However, in asphyxia the rise in blood pressure is more important in slowing the heart." Berezin (15) working with rabbits and large fish (pike), and Wiggers (16) using the brain of decapitated dogs have found that adrenalin in higher concentrations than are necessary to produce proportional peripheral results produce a constriction of cerebral vessels.

One may postulate that adrenalin by constricting the cerebral vessels produces asphyxia of the center and hence inhibition of the heart. I have found that asphyxia produces similar results previous to and after morphin. After morphin however, a slightly more pronounced slowing occurred toward the end of asphyxia than was produced before the use of the drug. In one case an actual acceleration occurred at the end of asphyxia.

Wiggers found that adrenalin in high concentration (1-500) was necessary to produce cerebral constriction. The solutions I have used were never greater than 1 cubic centimeter of 1-2000 and in the majority of the cases much more dilute so that if constriction were produced it would be very slight. The blood carries enough surplus oxygen to compensate for the deficiency in volume that would occur with constriction.

Since the greatest action of adrenalin on the vascular system is in the splanchnic area it is more than probable that the medulla and brain even if constriction of their vessels is effected receive a greater supply of blood after adrenalin than before it. I do not think that any possible degree of asphyxia which adrenalin might produce, and I am of the opinion that it does not produce any, would compare with the absolute artificial asphyxia effected in my experiments. And since no results were effected by total asphyxia I do not think the possible adrenalin asphyxia

could play any part; also if asphyxia were the causative agent I would expect slowing to occur in instances previous to morphin where only acceleration was effected.

I am sure that in no case when adrenalin was administered was there an acute lack of oxygen in the medullary centers.

That adrenalin produces no results when injected into the fourth ventricle, I think is due to the slow absorption from this region. It is only drugs that have a strong action on the central nervous system (strychnine) or able to destroy nervous tissue (chloroform, phenol) that produce quick results when injected into the fourth ventricle. Recently Meltzer and Auer (17) found that adrenalin injected intraspinally into the lumbar region is comparatively slowly absorbed, and Meltzer (18) found no absorption through nerves. Scott and Halliburton (19) on the contrary state that the injection of 1 cc. of 1-10,000 adrenalin into the sub-cerebellar cistern produces a rise in pressure as quickly as if injected intravenously. It is probable that a drug as quickly oxidized as adrenalin has not sufficient penetrating power to permeate directly the medullary substance. The absorption in different animals and man requires investigation.

CONCLUSIONS

1. Adrenalin has a direct central action and is synergistic with morphin.
2. Morphin to a degree sensitizes the vagus center.
3. The aggravated adrenalin action following morphin is due to the morphin sensitization and adrenalin-morphin synergism.
4. The increased blood pressure plays but a little part in effecting the inhibition of the heart.

REFERENCES

(1) Oliver and Schafer: Amer. Journ. of Physiol., 1895, xviii, 238.
(2) Brown: Journ. of Pharm. and Exper. Therap., 1916, viii.
(3) Cybulski: Centrlbl. f. Physiol., 1895, 172.
(4) Heinekamp: Journ. of Pharm. and Exper. Therap., 1919, xiv, 17.
(5) Cushny: Text-book of Pharmacology, p. 366, 6 ed.
(6) Cushny: Text-book of Pharmacology, p. 368, 7 ed.
(7) Meek and Eyster: Amer. Journ. of Physiol., 1915, xxxviii, 62.
(8) Hoskins and Lovelette: Journ. Amer. Med. Assoc., 1914, lxiii, 316.
(9) Stewart and Rogoff: Journ. of Pharm. and Exper. Therap., 1919, xiii, 127.
(10) Stewart and Rogoff: Amer. Journ. of Physiol., xlviii, 398.
(11) Jackson and Ewing: Amer. Journ. of Physiol., 1914, xxxiii, Proc. XXX.
(12) McGuigan and Ross: Journ. Pharm. and Exper. Therap., 1915, vii, 385.
(13) Sollmann: Manual of Pharmacology, 1917, 217.
(14) Starling: Principle of Phys., 1915, p. 984, et seq.
(15) Berezin: Journ. Amer. Med. Assoc., 1916, lxvii, 844. Ref.
(16) Wiggers: Amer. Journ. of Physiol., 1914, xlviii, 109.
(17) Auer and Meltzer: Amer. Journ. of Physiol., 1918, xlvii, 287.
(18) Meltzer: Rockefeller Institute, 9, 1909.
(19) Scott and Halliburton: Amer. Journ. of Physiol., 1912, xliv, 7.

THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

V. CURARA

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Langley and his pupils (1), (2), (3) have shown that curara paralyses many groups of preganglionic fibers by acting upon the peripheral nerve cells. In a recent paper Langley (4), working with cats, gives additional instances in which curara paralyses preganglionic fibers and concludes that it has a more or less paralysing action on all preganglionic nerves. The paralysing action of curara can be overcome by nicotine in sufficient amount. He includes among the fibers paralysed by curara the adrenaline secreting fibers. Although the paper does not appear to contain actual observations in support of this conclusion[1] the assumption is an extremely probable one. It is easy to show that it is correct. Having planned to use curara to paralyse the motor nerves in certain experiments on the epinephrin output, we were compelled to examine the question whether the doses required for this purpose would affect appreciably the adrenal secretory fibers. We found that this was the case and that curara could not be used in the proposed experiments.

Our results show that the epinephrin output in the cat is markedly diminished by the doses necessary to cause and to

[1] In one place Langley says "On the basis of these facts it follows that curari paralyses the adrenaline secreting fibers, and that this paralysis can be overcome by nicotine." The facts referred to are certain observations on the eye, which, so far as we can see, have no direct bearing on the action of curara upon the adrenal nerves, but which may be interpreted as showing that *if* curara paralyses the adrenaline secreting fibers, the paralysis can be overcome by nicotine.

maintain complete paralysis of the skeletal musculature even for a relatively short time and the diminution may be still quite definite when stimulation of the cardioinhibitory fibers, if they have been paralysed by the dose of curara, is again effective. We did not attempt to determine exactly the relative susceptibility of the epinephrin-secreting fibers and of the other groups of autonomic fibers as this had no bearing on the subject of our investigation. But since the cardio-inhibitory fibers are the most easily paralysed by curara of all the groups, according to Langley (4), we do not think that the adrenaline secreting fibers are correctly placed by him among the fibers which are paralysed with the greatest difficulty. His paper does not show on what evidence this classification of the adrenal fibers is based.

Our experiments were made on cats and with the technique described in previous papers (5), the epinephrin in adrenal vein blood collected from a pocket of the vena cava being assayed on rabbit intestine (and uterus) segments. The animals were anesthetized with urethane. The smallest dose of curara employed was 0.25 cc. of a 1 per cent solution per kilogram (0.75 cc. in a 3 kgm. cat), and the largest 0.5 cc. of the same solution per kilogram. The real dose would be somewhat greater as the abdominal aorta was tied at the bifurcation when the cava pocket was made. These doses caused complete paralysis of the skeletal muscles as shown by stimulation of the brachial nerve. The respiration ceased, but at the end of the short experiment spontaneous respiratory movements were observed after stopping the artificial respiration, and stimulation of the brachial nerve caused some contraction. Paralysis of the cardio-inhibitory fibers could be demonstrated but it was not lasting, and samples of adrenal vein blood collected after distinct recovery of the conductivity in the cardio-inhibitory path showed still a marked diminution of the epinephrin output.

A few samples of tracings used in the assay and protocols of the experiments follow.

Condensed protocol. Cat 378, male, weight, 3.07 kgm. Anesthetized with urethane

10.30 a.m. Cannulae inserted in trachea, external jugular vein and carotid artery; left vagus ligated and cut.

10.55 a.m. Cava pocket completed. Collected adrenal blood.

10.55 a.m. First specimen, 1.6 gram in 30 seconds (3.2 grams per minute). 10.55½ a.m. Second specimen, 5.35 grams in 120 seconds (2.7 grams per minute).

11.05 to 11.15 a.m. Blood pressure 120 mm. mercury. In this interval vagus stimulated 3 times. Stimulation caused marked fall of blood pressure and slowing of heart.

11.15 a.m. Started artificial respiration and began injection of curara.

11.15½ a.m. End of intravenous injection of 0.75 cc. of curara (1 per cent in physiological salt solution). Blood pressure 68 mm., falling during the next minute to 58 mm. mercury.

11.17 a.m. Cessation of spontaneous respiration. Vagus stimulation had no effect on blood pressure or heart rate.

11.18 a.m. Third adrenal specimen, 0.75 gram in 30 seconds (1.5 gram per minute).

11.18½ a.m. Fourth adrenal specimen, 4.7 grams in 210 seconds(1.34 gram per minute).

11.22 a.m. Blood pressure 78 mm. mercury.

11.22 to 11.26 a.m. Vagus stimulated 3 times. Caused a moderate fall of blood pressure and with stronger stimulation cessation of the heart beat.

11.26 a.m. Blood pressure 53 mm. mercury.

11.35 a.m. Stimulation of peripheral end of brachial nerve caused contraction of muscles. Spontaneous respiration not yet returned.

11.36 a.m. Fifth adrenal specimen, preliminary, collected for 60 seconds.

11.37 a.m. Sixth adrenal specimen, 1.7 gram in 360 seconds (0.3 gram per minute).

11.43 a.m. Palpation of abdominal aorta during vagus stimulation showed slowing and weakening of heart beat. Obtained indifferent blood from the cava (after tying off the adrenal veins). During the collection of this blood gasping movements were observed. Exposed heart and stimulated vagus; the auricles almost stopped and the ventricles were slowed. Combined weight of adrenals 0.518 gram.

The second adrenal blood specimen, collected before injection of curara, was shown to be much stronger than 1:6,600,000 adrenalin, decidedly stronger than 1:5,300,000. (fig. 1, observations 10,14 and 16, confirmed by other observations not reproduced), much weaker than 1:2,700,000 (observation not reproduced), somewhat weaker than 1:4,000,000 (fig. 1, observation 12). It was finally assayed at 1:4,500,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.0002 mgm. per kilogram of body weight per minute, not far from the average normal output under our experimental conditions.

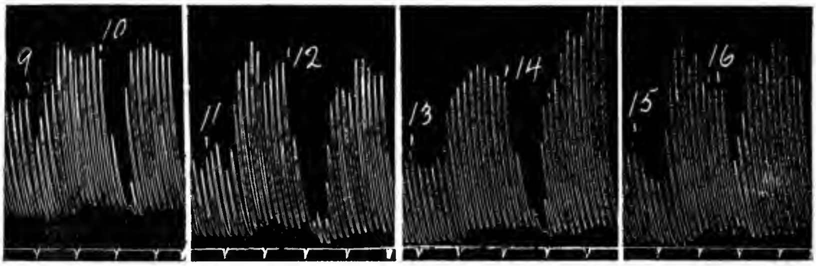

FIG. 1. INTESTINE TRACINGS. BLOODS FRON CAT 378

At *9*, *11*, *13*, and *15* Ringer was replaced by jugular blood, and this at *10* by jugular blood to which was added adrenalin to make a concentration of 1: 5,300,-000; at *12* by jugular blood to which was added adrenalin to make a concentration of 1: 4,000,000; at *14* by ths second adrenal blood specimen (collected before injection of curara); at *16* by jugular blood to which was added adrenalin to make a concentration of 1: 6,600,000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to two-thirds.)

The fourth adrenal specimen, collected $3\frac{1}{2}$ minutes after completion of the curara injection and about $1\frac{1}{2}$ minutes after paralysis of respiration was seen to be complete, was weaker than 1:5,300,000 adrenalin, decidedly stronger than 1:8,000,000 (fig. 2, observations 22 to 26, confirmed by observations 28 to 36). Two duplicate sets of observations are reproduced in figure 2 to illustrate a point in the technique of these intestine segment assays. The segment was beating more strongly when the lower row of tracings was taken, but it will be noted. that this does not at all disturb the relative magnitude of the inhibitory reactions. Two separate observations (not reproduced)

indicate that the 4th specimen was slightly weaker than 1:6,600,000. It was taken at 1:6,700,000, corresponding to an output of 0.0002 mgm. per minute for the cat, or 0.000065 mgm. per kilogram per minute, about one-third of the original output.

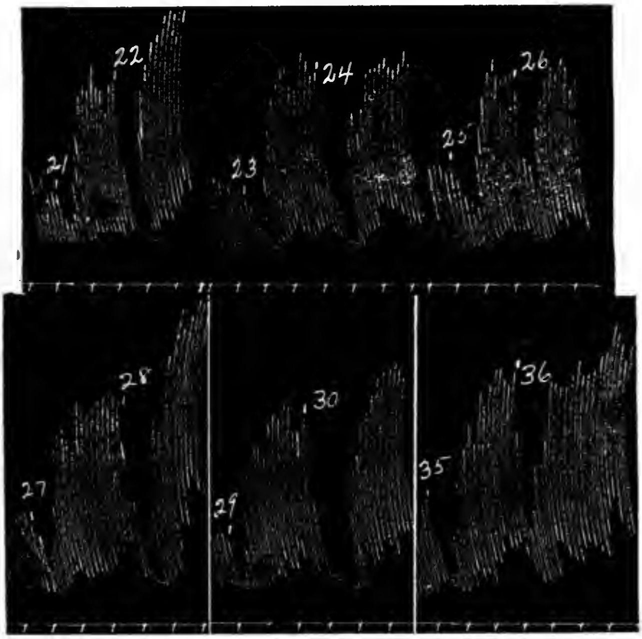

Fig. 2. Intestine Tracings. Bloods from Cat 378

At *21*, *23*, *25*, *27*, *29*, and *35* Ringer was replaced by indifferent (venous) blood (collected after injection of curara), and this at *22* and *28* by the fourth adrenal blood specimen (collected 3 minutes after injection of curara); at *24* and *30* by the indifferent blood to which was added adrenalin to make a concentration of 1: 5,300,000; at *26* and *36* by the indifferent blood to which was added adrenalin to make a concentration of 1: 8,000,000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to one half.)

The sixth adrenal blood specimen, procured $22\frac{1}{2}$ minutes after the injection of curara, was much weaker than 1: 2,700,000 (fig. 3, observations 46 and 48, confirmed by another set of observations not reproduced), and stronger than 1: 4,000,000 (fig. 3, observation 44). It was assayed at 1: 3,400,000, corre-

sponding to an output of 0.00009 mgm. per minute for the animal, or 0.00003 mgm. per kilogram per minute, not one-sixth of the original output. Although the blood flow at the time of collection of the sixth specimen was greatly reduced this reduction was not accompanied by a correspondingly increased concentration, as would be the case in an animal under urethane alone. The concentration is far below the possible maximum. There is no question then that curara produced a marked degree of paralysis of the epinephrin secretory path, which had not yet reached its maximum at a time when paralysis of the skeletal

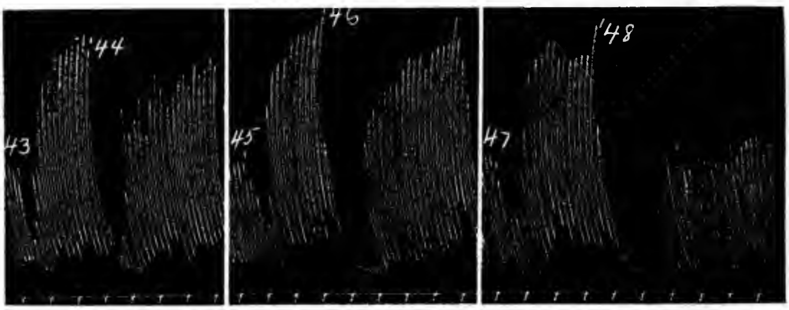

Fig. 3. Intestine Tracings. Bloods from Cat 378

At *43*, *45* and *47* Ringer was replaced by indifferent (venous) blood (collected after injection of curara) and this at *44* by the indifferent blood to which was added adrenalin to make a concentration of 1: 5,000,000; at *46* by the sixth adrenal blood specimen (collected 22 minutes after injection of curara); at *48* by the indifferet blood to which was added adrenalin to make a concentration of 1: 2,700,-000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to one-half.)

muscles was complete and at a time when the paralysis of the cardio-inhibitory fibers was wearing off.

The question might be raised whether the low blood pressure during collection of the sixth specimen might not be a factor in continuing and deepening the paralysis of the adrenal secretory fibers. We have abundant evidence that in an animal under urethane alone such an effect is not produced even when the blood flow through the adrenal and the blood pressure are still smaller than in this case. But it may be asked whether the paralysing action of curara might not be intensified by the

unfavorable influence of the low blood pressure. We have no evidence as to this and can only point out that the cardio-inhibitory peripheral cells, which are equally exposed to adverse effects of the low blood pressure, seem to have been recovering at a time when no recovery could be noted in the epinephrin secretion. It ought to be remarked that we were studying the influence of curara upon the spontaneous liberation of epinephrin, and a comparison of the effect produced by a given dose of the drug upon the conductivity to impulses originated by artificial stimulation of the preganglionic fibers in the one case and upon the conductivity to impulses originating without artificial stimulation in the central mechanism in the other case, may not be formally correct. The matter is of no importance for our purpose, which was accomplished as soon as it became evident that the epinephrin secretory fibers were among those most easily affected by curara and not among those most refractory to the drug. For this reason also it was of no importance to us to determine the relative ease of total paralysis of the adrenal path, although our experience with nicotine makes it probable that a moderate increase in the dose would bring us to the point where the epinephrin output would be undetectable with the test objects employed. Also it did not seem worth while to verify the ultimate restitution of conductivity with the smaller doses, as in the case of nicotine, since it could be assumed that this would occur, if the circulatory conditions remained favorable.

In the next experiment (cat 377) the dose per kilogram of bodyweight was somewhat increased, to about 0.4 cc. of the 1 per cent solution. Respiratory movements ceased promptly and collection of adrenal blood was begun within 20 seconds of the end of injection of curara, in order to see whether any transient stimulating action on the epinephrin secretion could be detected, as with nicotine.

Condensed protocol. Cat 377, female, weight, 2.7 kgm.

9.20 a.m. Anesthetized with ether. Inserted tracheal and jugular cannulae. Obtained indifferent blood from external jugular vein.

10.00 a.m. Cava pocket completed. Started artificial respiration. Collected adrenal blood.

10.00 a.m. First specimen, 4.6 grams in 30 seconds (9.2 grams per minute).

10.00½ a.m. Second specimen, 9.95 grams in 90 seconds (6.6 grams per minute).

10.05⅔ a.m. End of intravenous injection of 1.0 cc. curara solution (1 per cent in physiological salt solution).

10.06 a.m. Third adrenal specimen, 4.05 grams in 30 seconds (8.1 grams per minute).

10.06½ a.m. Fourth adrenal specimen, 7.5 grams in 90 seconds (5.0 grams per minute).

10.08 a.m. Fifth adrenal specimen, 5.8 grams in 120 seconds (2.9 grams per minute).

10.18 a.m. Cat died. Combined weight of adrenals 0.367 gram.

The assay on rabbit intestine segments showed that the second adrenal blood specimen, collected before injection of curara, was stronger than 1:8,600,000 adrenalin, stronger than 1:7,000,000, decidedly weaker than 1:4,300,000, somewhat weaker than 1:5,700,000. Taking the concentration at 1:6,000,000 we get 0.0011 mgm. epinephrin per minute for the cat, or 0.0004 mgm. per kilogram per minute. This output is within the normal range, though higher than the average. The 5th specimen, collection of which was begun 2⅓ minutes after completion of the curara injection, was much weaker than 1:5,700,000 adrenalin, and certainly no stronger than 1:14,000,000, probably somewhat weaker. Since the blood flow during collection of the 5th specimen was less than half as great as for the second, this alone shows that the output was much reduced by the curara. Taking the fifth specimen at 1:14,000,000 epinephrin, we get 0.0002 mgm. per minute for the cat, or 0.000075 mgm. per kilogram per minute, not one-fifth of the original output before curara. The fourth specimen, collection of which

was begun less than a minute after the curara injection had been completed, had a concentration of epinephrin not very different from that in the 5th specimen, if anything somewhat greater, but far less than in the second. It was shown to be much weaker than 1:5,700,000 and probably somewhat weaker than 1:14,000,000. Since the blood flow for the fourth was 70 per cent greater than for the fifth specimen, the curara paralysis of the epinephrin secretion, although quite evident at the time of collection of the fourth specimen, the output being reduced to one-third to one-fourth of the original output, had not reached its maximum and was still developing.

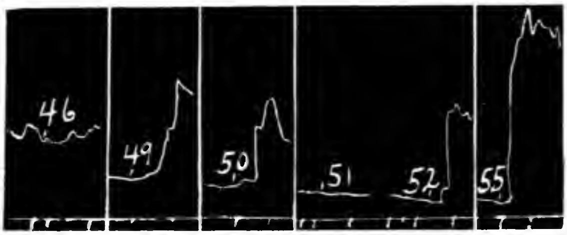

FIG. 4. UTERUS TRACINGS. BLOODS FROM CAT 377

At *46* Ringer was replaced by indifferent (venous) blood (collected after injection of curara); at *49* by the fifth adrenal blood specimen (collected $2\frac{1}{2}$ minutes after injection of curara); at *50* by the third adrenal blood specimen (collected immediately after injection of curara); at *51* by jugular blood (collected before injection of curara); at *52* by the fourth adrenal blood specimen (collected 1 minute after injection of curara); at *55* by the second adrenal blood specimen (collected before injection of curara). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds).

As so many intestine tracings have been reproduced to illustrate the assay on the bloods from cat 378, only a few uterus tracings from bloods of cat 377 are given (fig. 4). They show qualitatively that the second specimen is much stronger than the other adrenal bloods and that these cause a decidedly greater increase of tone than indifferent blood. It was also confirmed by the uterus that the second specimen did not differ much from a 1:5,700,000 concentration of adrenalin in the indifferent blood.

The largest dose of curara given was to cat 376, about double the dose per kilogram used for cat 378.

Condensed protocol. Cat 376, female, weight, 2.16 kgm. Anaesthetized with urethane

9.40 a.m. Inserted tracheal and jugular cannulae and obtained indifferent blood from external jugular vein.
10.08 a.m. Cava pocket completed. Collected adrenal blood.
10.08 a.m. First specimen, 0.5 gram in 30 seconds (1 gram per minute).
10.08½ a.m. Second specimen, 4.15 grams in 240 seconds (1.04 gram per minute).
10.18 a.m. End of intravenous injection of 1.0 cc. of curara solution (1 per cent in physiological salt solution).
10.20 a.m. Cessation of spontaneous respiration. Started artificial respiration.
10.20 a.m. Third adrenal specimen, 0.45 gram in 45 seconds (0.6 gram per minute).
10.20¾ a.m. Fourth adrenal specimen, 3.55 grams in 360 seconds (0.6 gram per minute).
10.30 a.m. Stimulation of peripheral end of brachial nerve caused contraction of muscles (toe movements).
10.40 a.m. Fifth adrenal specimen, preliminary, collected for 60 seconds.
10.41 a.m. Sixth adrenal specimen, 1.9 gram in 360 seconds (0.3 gram per minute).
Obtained another specimen of indifferent blood from the cava (after tying off the adrenal veins). Respiratory gasps during collection of this blood. Combined weight of adrenals 0.472 gram.

The second adrenal specimen, procured before injection of curara, was found to be decidedly weaker than 1:2,850,000 adrenalin, weaker than 1:4,300,000 (confirmed by several observations), much the same as 1:5,700,000 (confirmed by 4 sets of observations). Taking it at 1:5,700,000 we get 0.00018 mgm. per minute for the cat, or 0.00008 mgm. per kilogram per minute, considerably less than the average output. The fourth specimen, collection of which was begun 2¾ minutes after completion of the curara injection, was much weaker than 1:10,000,000, weaker than 1:11,400,000, no stronger than 1:14,000,000. Taking it at 1:14,000,000 we get an epinephrin output of 0.00004

mgm. per minute for the cat, or 0.00002 mgm. per kilogram per minute, only one-fourth of the original output before curara. The sixth adrenal specimen, collection of which was begun 23 minutes after injection of curara, was found to be much weaker than 1:2,850,000, stronger than 1:7,000,000, somewhat weaker than 1:4,300,000. Taking it at 1:5,000,000 we get an output of 0.00006 mgm. per minute for the cat, or 0.00003 mgm. per kilogram per minute. With the small blood flow a much greater concentration of epinephrin would have been expected in the sixth specimen, in the absence of curara. The paralysis was, therefore, still very evident at this time. The assay was not good enough to permit the conclusion that the output had recovered somewhat at the time of collection of the sixth specimen as compared with the fourth.

The ease with which curara depresses the epinephrin output raises the question whether the transient diminution in the output sometimes observed by us after strychnine (5), preceding the marked and long-lasting augmentation caused by that drug, may not be due to a transient paralysis of peripheral nerve cells on the efferent secretory path, since Langley has shown that strychnine, like curara, has a very general paralysing action on such nerve cells. However, the doses of strychnine employed by Langley to obtain paralysis of other groups of preganglionic fibers are enormous in comparison with the doses whose effect upon the epinephrin secretion we have studied.

SUMMARY

1. Curara in doses sufficient to paralyse the skeletal muscles in the cat markedly depresses the output of epinephrin from the adrenals. The depression begins promptly and may be still well marked when paralysis of the muscles has begun to wear off. While no attempt was made to compare exactly the doses required to paralyse the epinephrin-secretory fibers and the cardio-inhibitory fibers, a marked diminution in the epinephrin output was observed in samples of blood collected from

the adrenals at a time when stimulation of the vagus caused inhibition of the heart.

2. In general, curara should not be employed in experiments on the epinephrin output.

REFERENCES

(1) Langley and Dickinson: Journ. Physiol., 1890, xi, 509.
(2) Langley and Anderson: Journ. Physiol., 1892, xiii, 460.
(3) Langley: Journ. Physiol., 1911, xliii, 125.
(4) Langley: Journ. Physiol., 1918, lii, 247.
(5) Stewart and Rogoff: Journ. Pharm. and Exp. Therap., 1919, xiii, 95.

COCAINE INTOXICATION IN THE RABBIT

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In the course of experiments reported elsewhere (1) relative to the presence or absence of a secretory function of the sympathetic nerves to the thyroid gland, as indicated by prolonged poisoning by cocaine, a new light was thrown on the ability of rabbits to withstand such treatment. It has been thought that cocaine is only slowly eliminated from the body and that cumulative effects are very liable to occur if dosages be repeated oftener than once a day. Grode (2) working on rabbits, guinea-pigs, cats and dogs, decided that indications of cumulative effects were produced by daily injections of cocaine and sometimes after injections repeated only every other day. He could not detect any increased tolerance for the drug on repeated administration to the above named animals. Wiechowski (3) determined the amount of cocaine excreted in the urine of rabbits and dogs after cocaine treatment and found that rabbits destroy the drug completely so that none is excreted unchanged, while dogs excrete a small percentage of the drug unchanged. He also found no indications of increased tolerance or cumulative effects with daily injections of the drug. The work reported here will show that rabbits, at least, can withstand cocaine injections repeated much oftener than once daily, in fact, one and one-quarter hours is sufficient as a time interval between moderate doses.

Cocaine hydrochloride in 0.9 per cent NaCl solution was administered to young growing rabbits by subcutaneous injection. The usual aseptic precautions were observed and no infections resulted. The cocaine solutions were sterilized by boiling, tests having been carried out which showed that boiling did not no-

ticeably diminish the activity of the drug. Holbrook (4) reports similar results as to the resistance of cocaine to boiling. It was found that 10 mgm. cocaine per kilo body weight of the animals was sufficient to produce a constriction of the ear vessels and dilation of the pupil lasting for about thirty minutes. One series of rabbits was given this dosage, the injections being repeated five to ten times daily for eleven days. No detrimental effects could be detected from the behavior, general appearance or rate of growth of the animals, so long as the injections were not repeated oftener than about one and one-fourth hours. In case the time interval between injections was lessened to forty-five to sixty minutes the rabbits became hyperexcitable and uneasy, but no convulsions resulted. Attempts to produce convulsions by still further shortening the period between injections were not carried out. In this series the animals were being given more than the convulsive dosage (estimated at 45 mgm. per kilo body weight for rabbits, by Grode (2) each day and still no detrimental effects could be observed.

A second series of rabbits was given seven to ten injections daily for four days, the dosage being gradually increased from 12 to 18 mgm. per kilo body weight. And now, even the four times the convulsive and twice the lethal dosage was being administered daily, the effects never became noticeably toxic unless the time interval between injections was shortened to less than one and one-fourth hours. In case the injections were repeated as rapidly as every forty-five minutes the rabbits became nervous and hyperexcitable toward evening but no convulsions were observed.

Apparently then the cocaine must be eliminated from the body or rendered inactive within one to two hours, since there seems to be no cumulative action with injections repeated every one and one-fourth hours. The next step, therefore, was to determine how long after an injection the cocaine effects could be detected. The pupil was made use of in these tests. 1–1000 epinephrin instilled into a normal rabbit's eye produces no dilation, even though the instillation be repeated every few minutes for hours. However, dilation will occur under such treatment

following cocaine injections which in themselves are much below the strength necessary for dilation. In all instances the adrenalin was instilled into one eye while the other was kept as control.

While the pupil receiving no adrenalin instillation had always returned to normal in one and one-half hours after cocaine injections, the other showed the presence of effective amounts of cocaine as long as thirteen hours after a single injection of 10 mgm. cocaine per kilo body weight. In one instance three successive injections of 35 mgm. per kilo body weight, repeated at one and one-half hour intervals, were given a rabbit, and pupil dilation under adrenalin instillation occurred after 25 hours. In another rabbit 40 mgm. per kilo body weight was given in a similar manner and its effects could still be demonstrated at the end of twenty hours.

Here, then, seems to be a considerable discrepancy in results: No cumulative effects are noted with repeated injections provided the injections are spaced at least one and one-quarter hours apart, thus indicating rapid elimination or destruction of the drug. On the other hand the sensitizing effect of the drug on the sympathetic endings in the iris could be detected many hours after the injection. These results would seem to indicate that the cocaine is rapidly removed from the circulating fluids, thus preventing cumulative effects from repeated injections, while the concentration in the tissue of the nerve endings is lowered much more slowly, thus maintaining the sensitization for several hours. In order to assume that the drug is eliminated so much more slowly from the nerve endings than from the blood it would also be necessary to assume an actual combination of some sort between the cocaine and nerve endings. We have as yet no conclusive evidence of the existence of such a combination, however. It might well be that the cocaine so alters the metabolism of the sensitive nerve tissue that recovery does not follow immediately upon the disappearance of the drug. Instances of this latter kind of effect are found frequently, as in the fatty changes of the liver, heart and kidneys, following chloroform poisoning, the injurious effects often lasting for weeks after the chloroform has all been eliminated.

Whether the drug actually is combined with the nerve tissue and slowly released into the blood thus giving the prolonged effects, or whether the oxidation of the tissue is affected in such a way that the effects persist long after the disappearance of the drug cannot be decided at present. However, cumulative effects are avoided, provided the injections are far enough apart in time to allow the drug concentration in the blood to be kept down. For cocaine, one and one-fourth hours seems to be sufficient for the interval between injections.

Summarizing, the results of the work may be stated as follows:

1. No cumulative effects from repeated injections of the cocaine are found unless the interval between injections is less than one and one-fourth hours, thus indicating rapid destruction or elimination of the drug from the blood.

2. Sensitizing effects of the cocaine on the iris were found to last as high as 25 hours, due either to slow elimination of the cocaine from the tissues affected, or to more persistent functional changes following the disappearance of the drug.

The experimental part of this work was carried on in the Laboratory of Pharmacology of the University of Chicago at the suggestion, and under the direction, of Dr. A. L. Tatum, for whose aid and suggestions I desire to express my sincere thanks.

REFERENCES

(1) Mills, C. A.: Am. Jour. Physiol.
(2) Grode, J.: Arch. f. Exp. Path., 1912, lxvii, 172.
(3) Wiechowski: Arch. f. Exp. Path. u. Pharm., 1901, xlvi, 155.
(4) Holbrook, D. M.: Jour. Am. Med. Assn., 1912, lviii, 721.

THE CONSTITUENTS OF LATHYRUS SATIVUS SEEDS AND THEIR ACTION

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In 1917, Stockman (1) described the isolation of a toxic alkaloid from the seeds of Lathyrus sativus. He found that, in frogs, it produced paralysis of the motor nerve endings, and in mammals, a motor paresis referable to an action partly on the peripheral nerves and partly on the central nervous system. The present paper gives particulars of further experiments on the toxicity of lathyrus seeds.

Lathyrus seeds contain abundance of starch, the grains being irregularly round or oval with a distinct hilum and definite concentric rings. Cane sugar is also present. Extraction with saline solution yields albuminous constituents which, by salting out, may be separated into legumin, vicilin and legumelin. No evidence was found of a haemolytic or agglutinating body.

An extract of the seeds with absolute alcohol gives on evaporation a residue, part of which is insoluble in water. The insoluble portion can be separated into (a) a semi-fluid fixed oil, tinged with chlorophyll, and (b) a gum resin, soluble in ether and petroleum ether, insoluble in water but forming an emulsion with it, soluble in water containing sodium hydrate, which solution froths on shaking. When heated the gum-resin chars and evolves the odour of burning organic matter. If the watery emulsion be filtered the suspended resin remains behind and the filtrate on evaporation leaves a gummy residue. Injection of the emulsified fixed oil or gum-resin into frogs produced no effects.

Isolation of the active principle. As has been stated by Stockman the yield of alkaloid from the seeds is very small and

several methods of isolation were employed in an endeavor to obtain larger proportions of the active principle. The material was (a) extracted with 50 per cent and 90 per cent alcohol, expressed, the evaporated filtrate re-extracted with water and filtered, (b) macerated with boiling tartaric acid water, the filtrate evaporated, and the residue extracted with 90 per cent alcohol, (c) macerated with tartaric acid water and the filtrate treated with neutral lead acetate and sulphuretted hydrogen to remove part of the organic matter prior to evaporation and extraction with alcohol 90 per cent.

In all cases the toxic principles were isolated from the ultimate watery solutions by means of the usual Stas-Otto process. Comparison of the quantitative results by the different methods indicated that all were equally satisfactory, slight differences in the amounts yielded being readily accounted for by difficulties in the extraction.

Residue from group I (Stas-Otto method). This consists of a dark brown gummy material, almost insoluble in water and acidulated water. No glucoside is present. By extracting with petroleum ether a small amount of oil is recovered. The portion insoluble in petroleum ether is dark brown and resinous, soluble in alcohol, ether, chloroform and alkalies, a solution in dilute sodium hydrate froths on shaking. The bulk of this material appears to be an oleo-resin but mixed in this matrix are colourless plate-like crystals which are probably alkaloidal. The total residue amounts to 0.041 gram per 100 gram seeds.

A suspension of the residue was made in water by means of sodium bicarbonate and 0.1 gram was injected into the dorsal lymph sac of Rana esculenta. In fifteen minutes the animal was paretic, the legs being moved very slowly and weakly, the reflexes were not affected, the skin glands secreted very freely. Gradual recovery took place but even after twenty hours spontaneous movements were sluggish. One-tenth gram was again injected and within an hour spontaneous movements became gradually weaker, the legs ultimately being dragged, no alteration in reflex action was observed. Death took place during the night; post mortem, the spinal cord was inactive to faradism,

the voluntary nerves reacted only to strong faradic currents but the muscles showed no diminution in their excitability. Another frog injected with 0.2 gram exhibited the same symptoms and results.

In mice 0.1 gram injected subcutaneously produced in ten minutes slight paresis of the hind limbs which were dragged when the animal moved; the effect began to pass off in twenty minutes.

Action. The main action of the constituents of group I is the early production of paresis while the spinal reflexes are still active. Ultimately the spinal cord is paralysed and the excitability of the motor nerve endings lowered. It seems probable that the alkaloid is similar to that isolated in group II but that its action is masked by the presence of the oleo-resin, from which its separation was difficult.

Residue from group II (Stas-Otto method). There is obtained a light brown and gum-like residue which in parts shows irregular crystals, re-extraction with ether yielded a yellowish gummy residue which on exposure to air turned reddish. The residue is insoluble in water, readily soluble in water acidified with dilute HCl, and this solution gives dense precipitates with Mayer's reagent, phospho-molybdic acid, Bouchardat's reagent and gold chloride. By evaporating the acidified solution the alkaloidal hydrochlorate is obtained in the form of colourless irregular crystals.

An injection of 0.06 gram alkaloidal hydrochlorate into the dorsal lymph sac of Rana esculenta produces the following effects:

5 minutes. Lethargic, springs well but incoördinately, fore limbs appear weak.

10 minutes. Respiration ceased, movements incoordinate, spinal reflexes and conduction through cord augmented.

15 minutes. Does not spring, spinal reflexes increased, cornea almost insensitive.

20 minutes. Voluntary movement ceased, spinal reflexes increased, cord conduction good.

25 minutes. Spinal reflexes becoming weaker, corneal reflex absent, pupils dilated.

100 minutes. Spinal reflexes extremely weak.

240 minutes. Spinal reflexes absent.

300 minutes. Heart ceased, ventricle in systole, auricles in diastole, latter still respond to mechanical stimulus; spinal cord inactive to faradism, sciatic nerves insensitive to faradism, voluntary muscles active but reduced in excitability.

Injection of smaller doses (0.02 and 0.03 gram) produced in twenty minutes motor excitement and increased reflex responses; with the larger dose weakening of the movements of the hind limbs followed.

A mouse injected with 0.03 gram alkaloidal hydrochlorate exhibited weak and tremulous movements of the limbs which were also dragged; this persisted for two hours.

Action. There is produced first a depression of the higher nervous centres resulting in lethargy, incoördinate movement and finally cessation of voluntary movement; the corneal reflex is lost early and respiration stops. The reflex activity of the spinal cord is at first increased, later diminished, and then abolished; the peripheral voluntary nerve endings are paralysed and the excitability of the muscles lowered.

Residue from group III (Stas-Otto method). The residue, after purification, forms a gummy smear containing crystals in the form of colourless oblong plates; it tends to turn reddish on exposure to air and gives reactions similar to those of the alkaloid obtained in group II. The alkaloidal hydrochlorate forms colourless acicular and plate-like crystals arranged in rosettes.

Action. When injected into frogs or mice the effect is similar to that produced by the alkaloid of group II, with which this is apparently identical.

Residue from group IV (Stas-Otto method). This consists of a yellowish matrix containing colourless crystalline leaflets and plates. The residue is slightly soluble in water, imparting to it an alkaline reaction; an acid solution gives precipitates with alkaloidal reagents. The pure alkaloid is obtainable in needle and plate-like crystals and the hydrochlorate in needle-shaped crystals.

Action. The injection of 0.01 gram alkaloidal hydrochlorate into the dorsal lymph sac of Rana esculenta gives the following results:

5 minutes. Distinctly lethargic and paretic, when springing drags hind limbs which are drawn up slowly.
15 minutes. Still moves spontaneously, spinal reflexes more active, cord conduction good, respiration ceased, pupils dilated, eyelids closed, cornea insensitive.
25 minutes. Spinal reflexes diminishing, extension of hind limbs much weaker, no reflex from pinching skin of head, back and abdomen.
45 minutes. Spinal reflexes completely abolished, stimulation by faradic current over spine causes contraction of limb muscles, sciatic nerves and voluntary muscles both react to faradism, tracings taken of the muscular contraction show that the excitability is reduced and that the relaxation period is much prolonged; under repeated stimulation, the relaxation period becomes gradually shorter, see tracings 1, 2 and 3. Auricles and ventricles both ceased in diastole but respond to stimulation.

The following protocol was obtained from a mouse injected with 0.02 gram alkaloidal hydrochlorate:

15 minutes. Lethargic, spasmodic muscular twitchings occurring, movements of legs tremulous.
20 minutes. Movements weaker and tremulous, unable to support on hind legs.
35 minutes. Hind legs abducted, moves only when stimulated and then with tremors, cannot recover if laid on side, spinal reflexes diminished, respiration 100 per minute.
40 minutes. Respiration 72 per minute, weaker and dyspnoeic, frequent spasmodic twitching of muscles.
42 minutes. Respiration ceased, corneal reflex absent, respiratory convulsions follow. Post mortem—sciatic nerves active but inactive after one and one-half hours, muscles active.

A second mouse repeatedly injected with smaller doses showed the same paresis with slowing and weakening of the respiration.

Action. The alkaloid first produces a condition of paresis due to an action on some part of the brain since it occurs while

Fig. 1. Action of alkaloidal hydrochlorate (group IV) from Lathyrus sativus on muscle curve, frog's gastrocnemius, single stimulation, time tracing 1 second, coil 10 cms.

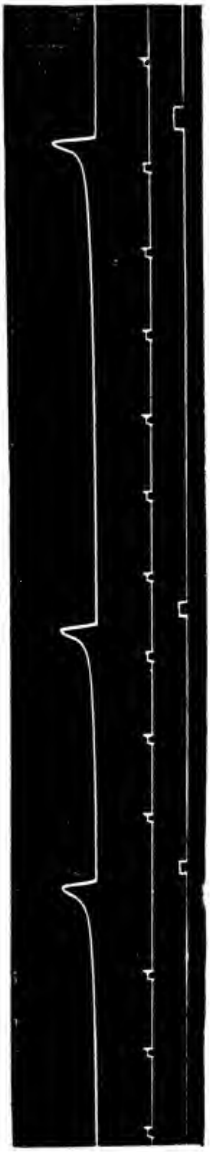

Fig. 2. Action of alkaloidal hydrochlorate (group IV) from Lathyrus sativus on muscle curve, frog's gastrocnemius, time tracing 1 second, coil 10 cms. Effect of repeated stimulation.

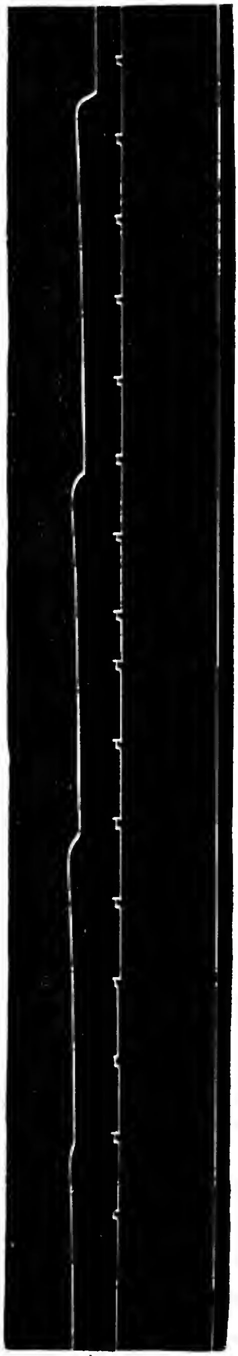

Fig. 3. Action of alkaloidal hydrochlorate (group IV) from Lathyrus sativus on muscle curve, frog's rectus femoris, time tracing 1 second, coil 10 cms.

the cord reflexes are augmented in the frog. Reflex activity is diminished from above downwards, commencing with the corneal reflex, and the diminution in the spinal cord seems to commence with a depression of the afferent portion of the reflex arc. The muscles are directly affected, excitability and contractility being diminished while the relaxation period is much prolonged; by repeated stimulation of the muscle the period of relaxation gradually shortens. This action resembles that of veratrine. The alkaloid also slows the heart rate and weakens the force of the beats. In mice the cause of death is respiratory paralysis but they also show that reflex activity is interfered with.

CONCLUSIONS

1. Lathyrus sativus seeds contain starch, cane sugar, legumin vicilin, legumelin, a fixed oil, a gum resin, and an oleo-resin, the last three in small amounts only.

2. The active principle of the seeds consists of two alkaloids which differ slightly in their action and are separable in group II and group IV of the Stas-Otto process. The percentage yield of alkaloid is extremely small, the maximum obtained of the first alkaloid being 0.0094 per cent and of the second 0.0035 per cent.

3. The characteristic action of both alkaloids in frogs is the early production of paresis due to an action on the central nervous system, probably in the brain. Later, both first increase and then diminish the reflex activity of the spinal cord.

4. The alkaloid from group II produces finally paralysis of the voluntary peripheral nerve endings followed by diminution and loss of the excitability of the voluntary muscular tissue. The alkaloid from group IV, on the other hand, has a more predominant action on the spinal cord where it appears to interfere first with the receptive side of the reflex arc. It does not appear to affect the voluntary nerve terminations but lowers the direct excitability of the muscle tissue and lengthens the relaxation period, the muscle curve being comparable to that of veratrine.

5. In one of the frogs injected with group II alkaloid the extensor muscles of the limbs, especially of the hind legs, became

rigid two hours after the injection. Postmortem the rigidity passed off but the muscles were then inexcitable electrically. Stockman observed flexor rigidity in monkeys (2) and rigidity and contracture of the leg muscles are commonly seen in men suffering from lathyrism (3).

6. Both alkaloids slow and weaken the heart beat in frogs and cause paresis in mice followed by death from respiratory paralysis.

7. The above results confirm the investigations of Stockman on the cause of the toxicity of Lathyrus seeds. It is agreed that for a complete pharmacological investigation of the active principles the isolation of the alkaloid on a large scale would require to be undertaken.

REFERENCES

(1) Stockman: Edinburgh Med. Jour., n. s., 1917, xix, no. 5, pp. 294–295. Full references to previous literature are given in this paper.
(2) Stockman: Edinburgh Med. Jour., n. s., 1917, xix, no. 5, pp. 285 and 287.
(3) Stockman: Lathyrism in Man. Edinburgh Med. Jour., n. s., 1917, xix, no. 5, pp. 297–307.

THE RESTORATION OF THE FROG'S HEART IN CHLOROFORM POISONING

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The problem which the experiments here recorded were intended to solve may be stated as follows:

A frog's heart being deeply depressed by chloroform, what drugs, if any, are able, in the continued presence of the chloroform, to cause more or less restoration of the heartbeat?

Especial weight is laid upon the continued presence of the chloroform, since it is already known that by perfusing with Ringer's fluid alone the chloroform can be washed away, so that the heart gradually resumes its activity.

Method. All frogs were pithed. The heart was perfused in situ through a canula inserted into the inferior cava, the perfusing fluid escaping by the cut aortas. The ventricular apex was attached to the writing lever so that the upstroke in the tracings represents systole. The pressure was maintained equable by perfusing from Marriott bottles. A frog's Ringer was used and in it the chloroform and the drugs to be tested were dissolved, the latter being added to the chloroform solution as soon as the chloroform depression was sufficiently pronounced. In all figures the time marker indicates thirty seconds.

It is important to notice that in using this method there is always a certain pressure in the sinus and auricle, hence the conditions are not identical with those which obtain when the mammalian heart with intact circulation is enfeebled or stopped by chloroform. In this case, when the heart fails, there is little or no return of blood to the right auricle and drugs introduced into the venous circulation may not reach the heart.

Adrenalin. The heart depressed to almost complete stoppage by perfusion with 0.03 per cent chloroform is restored with great rapidity on adding adrenalin 1: 1,000,000 to the chloroform solution (fig. 1) and continues beating with undiminished efficiency though still receiving a toxic concentration of chloroform. Figure 2 shows a perfusion with chloroform plus adrenalin continued for thirty minutes without any signs of diminished activity. The restoration on addition of adrenalin is remarkably quick, much

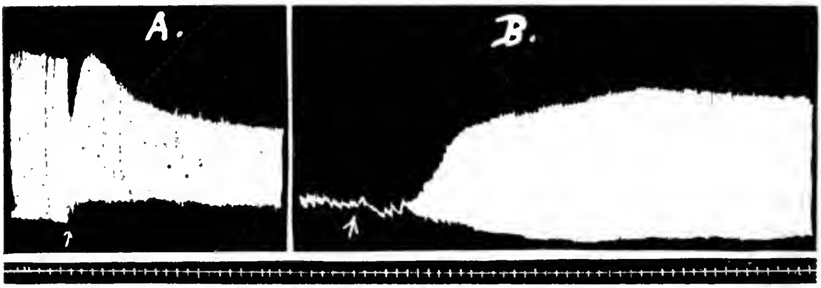

FIG. 1. *A*, at arrow $CHCl_3$ 0.03 per cent; *B*, at arrow $CHCl_3$, 0.03 per cent plus adrenalin 1: 1000,000. Between *A* and *B* 16 minutes.

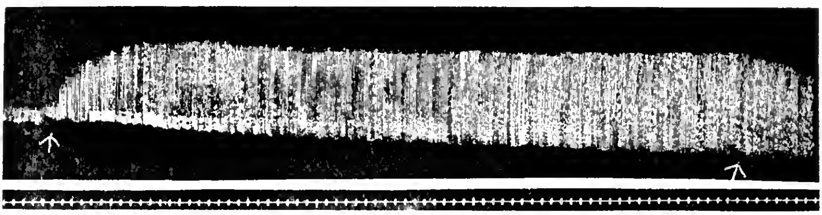

FIG. 2. Heart depressed by $CHCl_3$ 0.02 per cent. At first arrow $CHCl_3$ 0.02 per cent plus adrenalin 1:1000,000. At second arrow $CHCl_3$ 0.02 per cent alone.

more so than when the chloroform is withdrawn and the perfusion continued with Ringer's fluid alone. The comparatively gradual recovery under Ringer, indicating the difficulty of completely washing out the chloroform, is shown in figure 3 (same heart and same chloroform concentration as in figure 1).

Tyramin. The restoration on adding tyramin 1: 100,000 to the chloroform solution (fig. 4) is also extraordinarily quick and effective as compared with Ringer alone. As under adrenalin

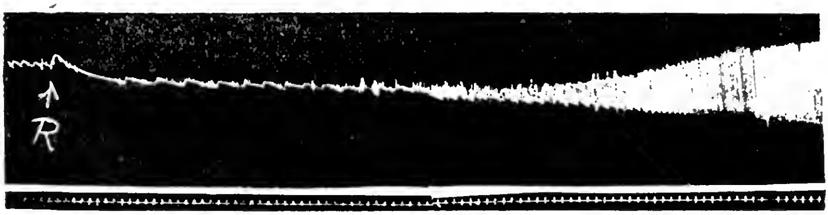

FIG. 3. Heart depressed by $CHCl_3$; at arrow Ringer only.

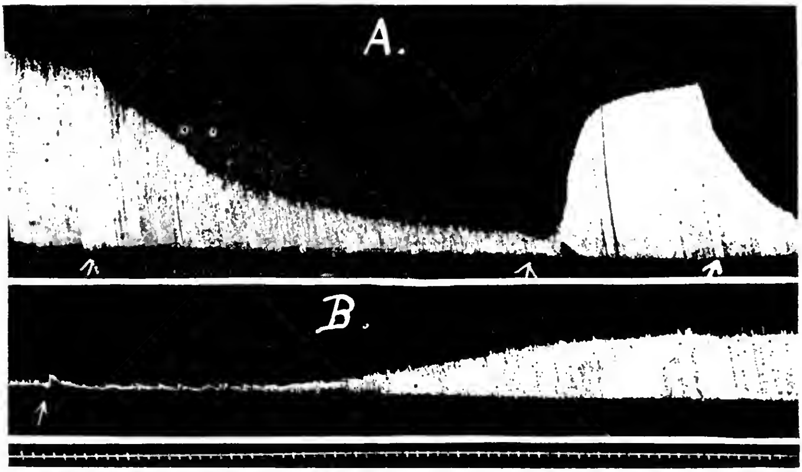

FIG. 4. *A*, at first arrow $CHCl_3$ 0.03 per cent; at second arrow $CHCl_3$ 0.03 per cent plus tyramin 1:100,000; at third arrow $CHCl_3$ 0.03 per cent alone. *B* continuation of *A*, at arrow Ringer only.

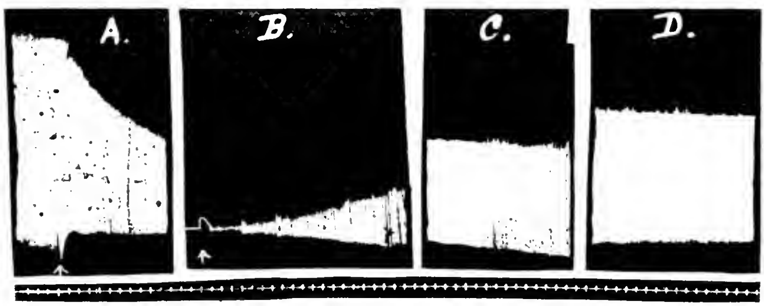

FIG. 5. *A*, at arrow $CHCl_3$ 0.03 per cent; *B*, at arrow $CHCl_3$ 0.03 per cent plus tyramin 1:500,000; *C* and *D* continuation of *B*. Between *A* and *B* 25 minutes; *B* and *C* 26 minutes; *C* and *D* 72 minutes.

the perfusion with tyramin plus chloroform may be continued for a long time without any evidence of deterioration in the heart. Figure 5 shows a heart still beating well after perfusion for nearly three hours with 0.03 per cent chloroform plus tyramin 1: 500,000. In this case the recovery was more gradual since the tyramin solution was considerably more dilute.

Pituitrin. The addition of 0.5 per cent pituitrin to the chloroform solution was found to have little or no restorative effect

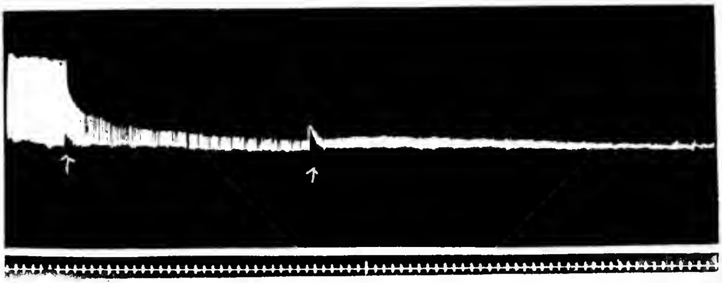

Fig. 6. At first arrow $CHCl_3$ 0.03 per cent. At second arrow $CHCl_3$ 0.03 per cent plus pituitrin 0.5 per cent.

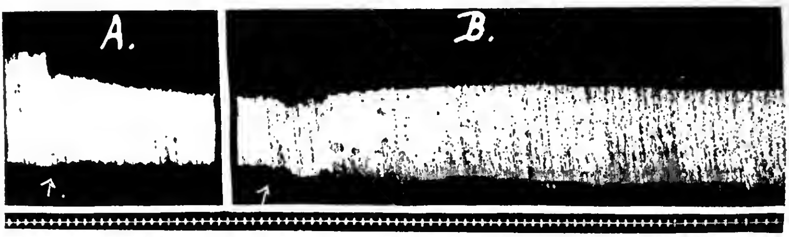

Fig. 7. *A*, at arrow $CHCl_3$ 0.02 per cent; *B*, at arrow $CHCl_3$ 0.02 per cent plus pituitrin 0.5 per cent. Between *A* and *B* 17 minutes.

(fig. 6). Even with light chloroform poisoning pituitrin was not able to completely restore the heart (fig. 7).

Strontium chloride. When about 0.1 per cent of strontium chloride is added to the chloroform solution the previous depression is quickly relieved but the improvement is not maintained, moreover there is evidence in the slowing that the strontium is itself affecting the heart (fig. 8). With greater concentration of strontium, 0.5 per cent, the toxic effects are

more marked and at the same time the antagonism to the chloroform depression is very evident (fig. 9).

Strophanthus. The solutions were made with tincture of strophanthus B.P. Strophanthus added to the chloroform solution has an effective restorative action but the rate at which this takes place is to a certain extent dependent upon the concentration of the strophanthus. With 0.01 per cent tincture of stro-

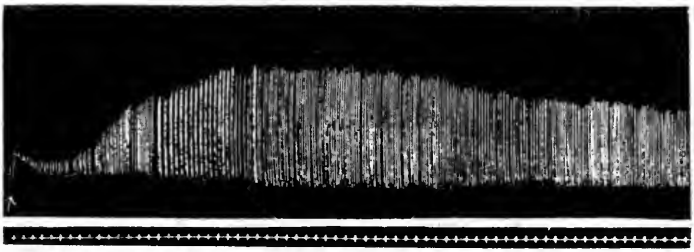

FIG. 8. Heart depressed by $CHCl_3$. At arrow $CHCl_3$ 0.03 per cent plus $SrCl_2$ 0.1 per cent.

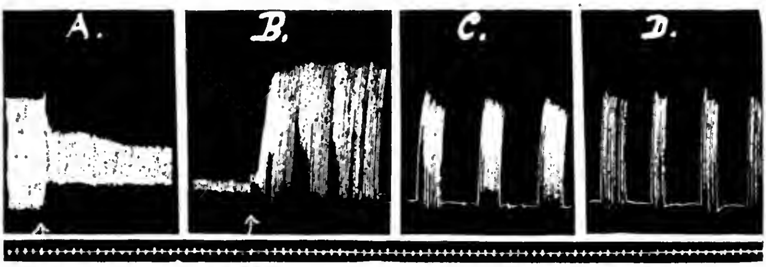

FIG. 9. *A*, at arrow $CHCl_3$ 0.03 per cent; *B*, at arrow $CHCl_3$ 0.03 per cent plus $SrCl_2$ 0.5 per cent. *C* and *D* continuation of *B*. Between *A* and *B* 16 minutes, *B* and *C*, 40 minutes, *C* and *D* 30 minutes.

phanthus (fig. 10) the restoration set in slowly and after 50 minutes was only about 50 per cent of the normal. With 0.025 per cent (fig. 11) the restoration was much quicker and at its best reached about 80 per cent of the normal, but under continued perfusion symptoms of strophanthus poisoning appeared. With 0.1 per cent strophanthus (fig. 12) the restoration was quick and so effective that in about 6 minutes the heart in the presence of chloroform was beating even better than before any chloroform

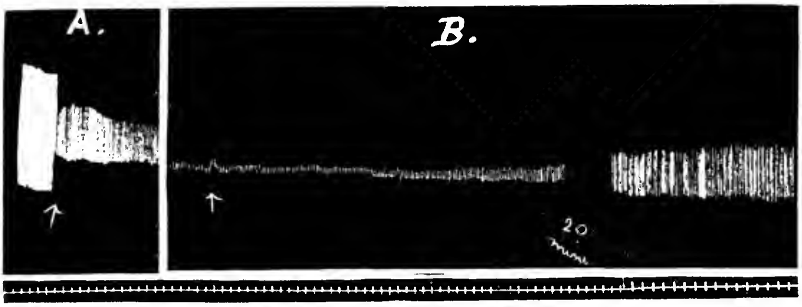

FIG. 10. *A*, at arrow $CHCl_3$ 0.03 per cent. *B*, at arrow $CHCl_3$ 0.03 per cent plus tincture of strophanthus 0.01 per cent. Between *A* and *B* 19 minutes.

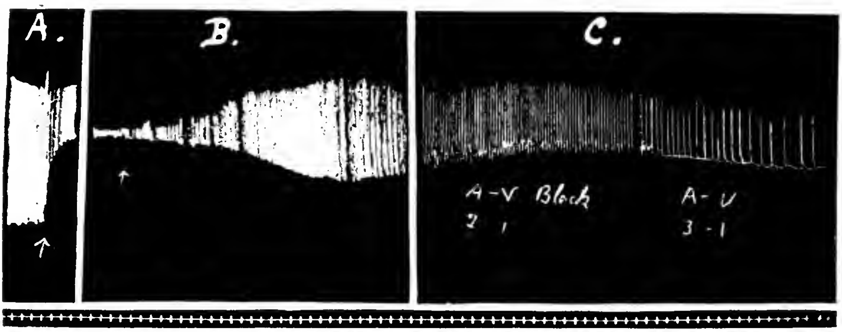

FIG. 11. *A*, at arrow $CHCl_3$ 0.03 per cent; *B*, at arrow $CHCl_3$ 0.03 per cent plus tincture of strophanthus 0.025 per cent. Between *A* and *B* 27 minutes, *B* and *C*, 5 minutes. *C* is continuation of *B*.

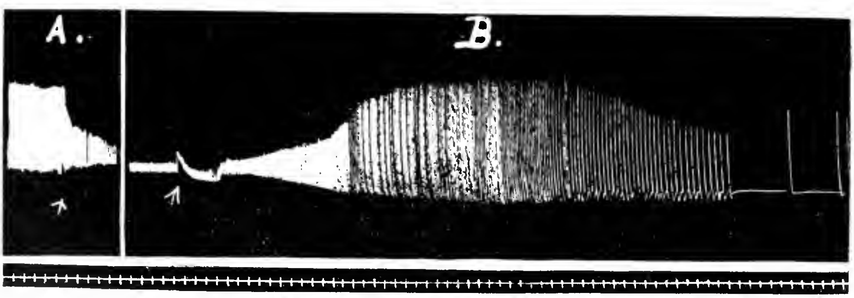

FIG. 12. *A*, at arrow $CHCl_3$ 0.03 per cent; *B*, at arrow $CHCl_3$ 0.03 per cent plus tincture of strophanthus 0.1 per cent. Between *A* and *B* 17 minutes.

had been applied; however toxic symptoms soon followed, the heart finally stopping. It may be noted here that when a normal heart is perfused with a powerfully depressant concentration of chloroform plus 0.02 per cent to 0.1 per cent tincture of strophanthus the characteristic death in middle position or in systole takes place in spite of the chloroform (fig. 18).

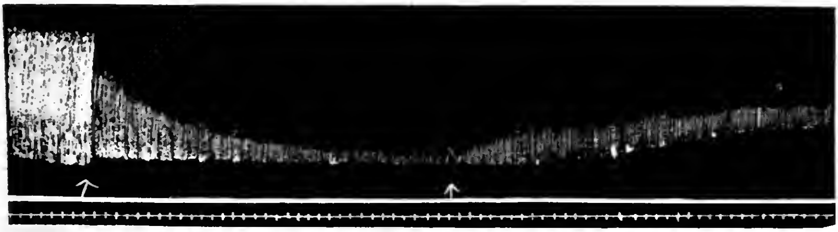

FIG. 13. First arrow $CHCl_3$ 0.03 per cent. Second arrow $CHCl_3$ 0.03 per cent plus caffeine 0.1 per cent.

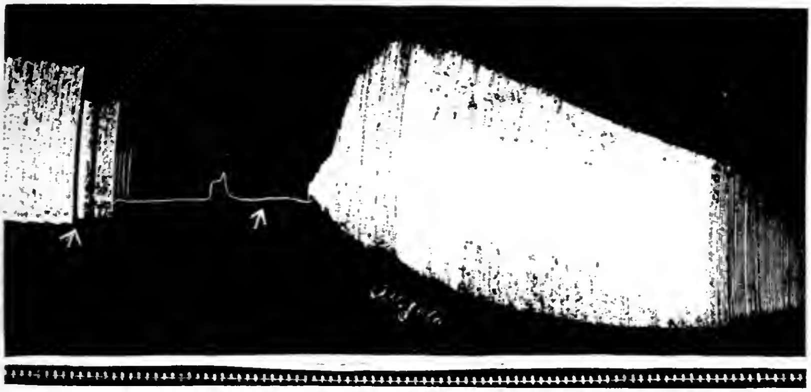

FIG. 14. First arrow $CHCl_3$ 0.04 per cent. Second arrow $CHCl_3$ 0.04 per cent plus caffeine 0.25 per cent.

Caffeine. After chloroform depression 0.1 per cent caffeine added to the chloroform solution has but very slight restorative effect (fig. 13); with 0.15 per cent the effect is a little better. Caffeine 0.25 per cent very quickly and completely restores the heart, which is even for a time actually better than before the chloroform (fig. 14), the improvement is however temporary if the perfusion is continued.

Diuretine. The chloroformed heart is hardly improved at all by the addition of 0.01 per cent diuretine to the chloroform solution but 0.025 per cent causes a very considerable restoration which is maintained for at least an hour (fig. 15). This concentration does not however completely relieve the chloroform depression, for on changing the perfusion to plain Ringer the heart is further improved. With 0.05 per cent there was marked

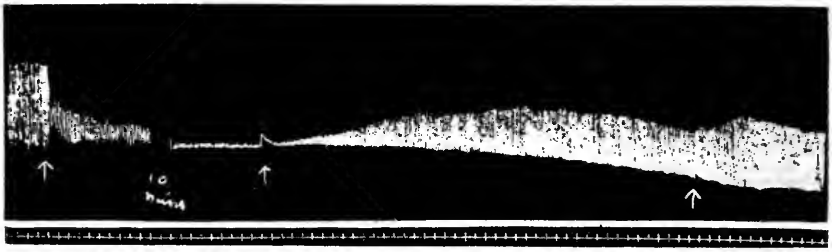

FIG. 15. First arrow $CHCl_3$ 0.03 per cent. Second arrow $CHCl_3$ 0.03 per cent plus diuretine 0.025 per cent. Third arrow Ringer only.

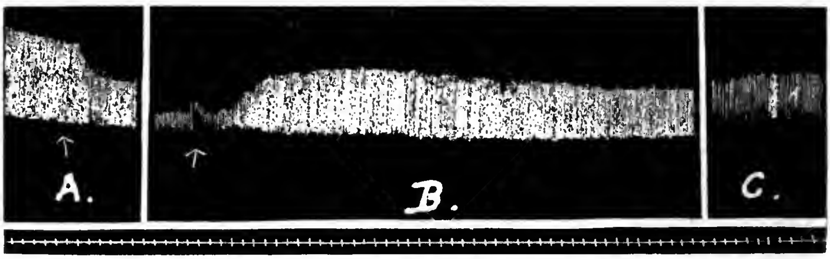

FIG. 16. *A*, at arrow $CHCl_3$ 0.03 per cent; *B*, at arrow $CHCl_3$ 0.03 per cent plus diuretine 0.05 per cent. Between *A* and *B* 28 minutes; *B* and *C* 22 minutes. *C* is continuation of *B*.

restoration which continued for at least thirty minutes (fig. 16). With 0.1 per cent diuretine there was very quick and complete restoration but the effect was quite temporary and was followed by sudden stoppage in diastole, such as can be produced by higher concentrations of diuretine (or caffeine) in normal hearts. On changing to plain Ringer, the heart resumed beating with at first distinct evidence of stimulation due doubtless to diminution of the toxic amount of diuretine in the heart (fig. 17). When a

fresh heart is perfused with a toxic concentration of chloroform plus one of the drugs (excepting pituitrin) employed in the above experiments, the chloroform depression is usually slight and more or less restoration follows quickly. As an example of what happens figure 18 shows the effect of chloroform plus strophanthus (0.02 per cent and 0.1 per cent) on a fresh heart; the chloro-

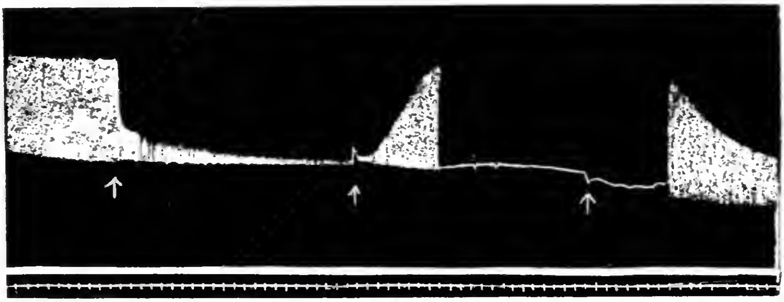

FIG. 17. First arrow $CHCl_3$ 0.03 per cent. Second arrow $CHCl_3$ 0.03 per cent plus diuretine 0.1 per cent. Third arrow Ringer only.

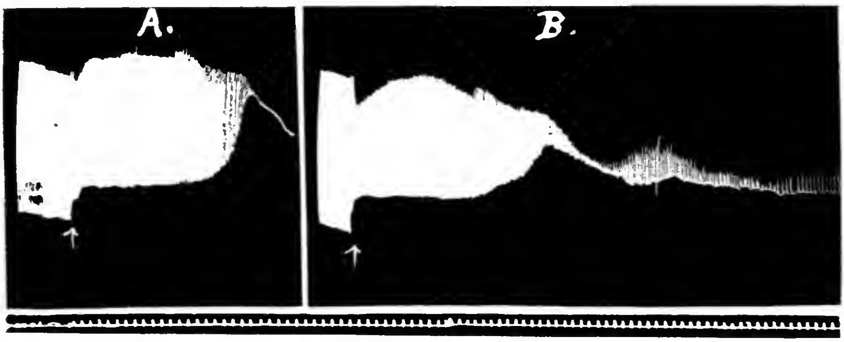

FIG. 18. *A*, at arrow $CHCl_3$ 0.03 per cent plus tincture of strophanthus 0.1 per cent; *B*, at arrow $CHCl_3$ 0.03 per cent plus tincture of strophanthus 0.02 per cent.

form depression is distinctly indicated but is then counteracted by the strophanthus which finally causes death in its characteristic way. In similar experiments pituitrin was as before the least effective of the group.

As has been noted above, perfusion with caffeine, diuretine, strophanthus or strontium chloride causes restoration followed

if the perfusion is continued by toxic symptoms. To get out of this difficulty a second set of experiments was made differing from the first in that, as soon as distinct evidence of restoration

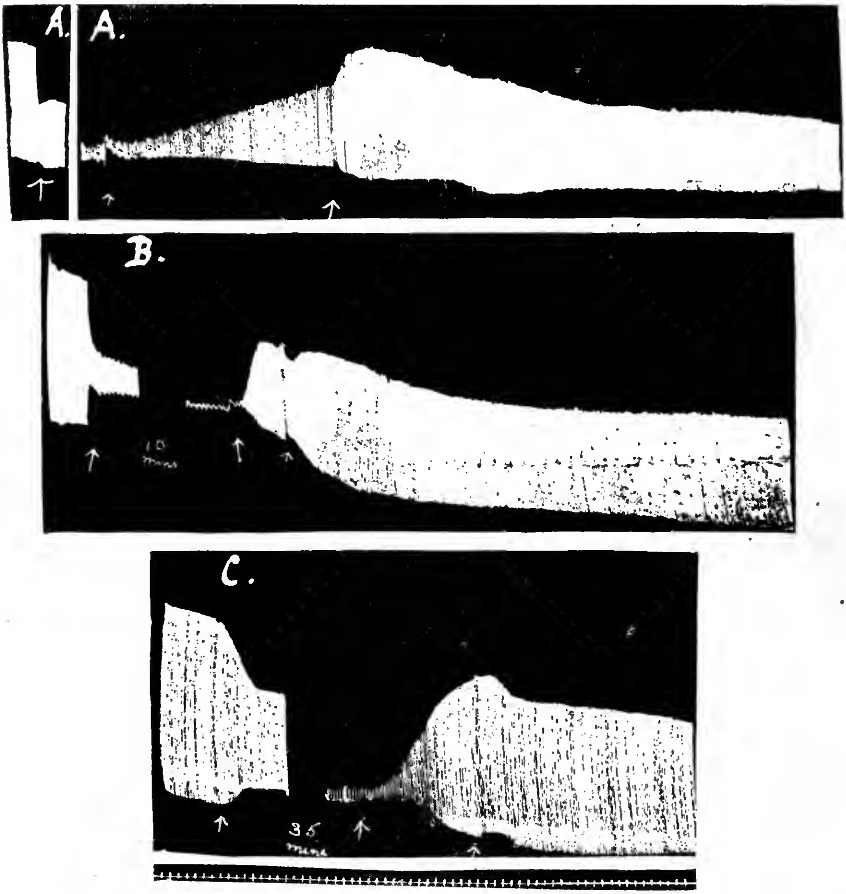

FIG. 19. *A*, *B* and *C*, at first arrow $CHCl_3$ 0.03 per cent. *A*, at second arrow $CHCl_3$ plus tincture of strophanthus 0.02 per cent; *B*, at second arrow $CHCl_3$ plus caffeine 0.25 per cent; *C*, at second arrow $CHCl_3$ plus adrenalin 1:1,000,000. *A*, *B* and *C*, at third arrow Ringer only. Fresh heart for each tracing.

was obtained, the perfusion fluid was changed to plain Ringer. Figure 19 shows that this method is perfectly successful and more efficient than simply washing away the chloroform with Ringer (compare figures 3 and 4).

Summary. It is shown in perfusion experiments that frogs' hearts depressed by chloroform may be restored to nearly or in some cases quite normal activity by adding to the chloroform solution small quantities of adrenalin, tyramin, strophanthus, diuretine, caffeine or strontium chloride (pituitrin failed). In the case of adrenalin and tyramin the restored hearts will continue beating well in presence of toxic amounts of chloroform for some hours. The other substances when added to the chloroform solution cause, first, restoration and then characteristic toxic symptoms of their own, but this latter difficulty may be got over if, as soon as restoration has taken place, the perfusion fluid is changed to plain Ringer. By this method of giving a small quantity of the antagonist and following with plain Ringer, restoration is much quicker than when the chloroform is simply washed away.

Application. It must be borne in mind that the antagonism shown in these experiments applies only to the effect of chloroform upon the heart muscle and that in order to be effectively used the antagonist must reach the heart. In so far as these results, obtained in certain experimental conditions with frogs' hearts, are applicable to warm-blooded animals they indicate the importance of detecting early symptoms of heart failure under chloroform—an injection of a suitable antagonist made at once would probably be successful, delay till failure had actually occurred might be fatal.

ANAPHYLACTOID PHENOMENA FROM THE INTRAVENOUS ADMINISTRATION OF VARIOUS COLLOIDS, ARSENICALS AND OTHER AGENTS[1]

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I. INTRODUCTION

The object of this study is to throw additional light on the dangers of intravenous medication and the relationship of these to anaphylaxis and anaphylactic shock by a detailed study of the effects of the intravenous administration of a variety of agents, principally colloids of non-protein origin. Before proceeding to the results of our experiments, a brief consideration of the fundamental aspects of the problem is desirable.

[1] This research was supported in part by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

The intravenous injection of certain agents such as serum, diphtheria antitoxin, foreign proteins and arsphenamine produce in some individuals disturbances which resemble the symptoms of anaphylactic shock. It is alleged that in these individuals a state of anaphylaxis exists or, at least, an unusual degree of sensitiveness, presumably the state of sensitization, although in many instances such individuals have not been previously treated with the agent. This is particularly true of such agents as arsphenamine, while with proteins, no matter what the source, sensitization might conceivably exist.

This is rendered less probable in view of the fact that similar responses, which may be conveniently designated as "anaphylactoid phenomena," occur after the injection of various nonprotein colloids into previously untreated or normal animals. Among such agents are kaolin, starch and inulin, and agar, previously investigated by Friedberger (1), Nathan (2) and Bordet (3), and Novy and DeKruif (4), respectively. For instance, agar, a nonprotein colloid and otherwise physiologically nontoxic and inert, when injected intravenously into a guinea-pig in the form of a sol-gel (1 : 6) in the very small dosage of 0.0094 mgm. agar per gram of animal produces symptoms indistinguishable from true anaphylactic shock and death and at autopsy the lungs are markedly distended. In fact, Novy, DeKruif and Novy (5) state that agar in this way is 33 times as toxic as peptone for the guinea-pig. From this fact, and, also, since proteins are colloids, it has been alleged (6) that the anaphylactoid symptoms, if not anaphylactic shock itself, are essentially a colloidal phenomenon. The explosiveness of the symptoms is said to be brought about by some disturbances in the aggregates of the colloids themselves, of the blood colloids and even in the cells free from blood and lymph. A review of the extensive work that has been done on anaphylaxis renders it evident that by leaning on this view exclusively it is difficult to reconcile the effects produced by certain substances with the state of true anaphylaxis and the phenomena of anaphylactic shock merely by the close resemblance of the symptoms. Solutions of arsphenamine have been brought into the category of colloids by Danysz (7) who claims

that the base is precipitated out by constituents of the blood, and again formed into soluble organic derivatives in the circulation. The disturbances accompanying arsphenamine injections are said to be associated with the rapidity of this precipitation, the rate depending on the alkalinity and peculiarities of individual plasma.

It was, therefore, resolved to investigate a variety of agents to see if these could be classed according to whether they possessed colloid characteristics or not. A number were found to act outwardly similar to agar. Finally, it was deemed necessary to investigate in particular the symptoms of lung inflation from agar and similar agents, for, if this could be substantiated with surviving lungs and if stimulation of smooth muscle in other regions could be demonstrated, namely, in the intestines and uterus, then indeed it would appear as if the explanation of "shock" on a colloidal basis had some foundation in fact. This might also explain the accidents and disturbances accompanying the intravenous administration of sundry agents, including arsphenamine.

With few exceptions, the tendency of the investigations concerning agents producing anaphylactoid phenomena appears to be limited almost exclusively to the symptomatology. Owing to its close relation to all physiological functions, and the respiratory function in particular, it is obvious that the circulation should not be left out of consideration. If precipitations in the blood stream occur, as alleged from in vitro experiments, these should be looked for microscopically and otherwise.

We have attempted to cover all these features. That is, the symptoms together with the changes at autopsy and microscopic examinations of the lungs, agglutination, and responses of surviving bronchial, intestine and uterine musculature have been taken into consideration. In this paper, the results of intravenous injections in guinea-pigs are recorded and the various agents classified. The remaining features will be presented in subsequent papers.

II. METHODS

Guinea-pigs of the average of 300 grams body weight were used throughout. A small skin incision was made, using a bare whiff of ether as anesthesia, for exposure of the jugular veins. The majority of injections were made into the left jugular vein, sometimes into the right vein. The injections were made slowly, consuming about sixty seconds for 3 cc. of solution. All agents were suspended or dissolved in 0.9 per cent NaCl and injected at about 38°C. Immediately after the injection, a small bulldog clip was placed on the vein, the animal was untied and observed. The volume of solution injected was 3 cc. as a rule, but this was varied from time to time to suit special purposes. Assuming that a 300-gram guinea-pig contains about 6 per cent of blood, or 18 grams, the volume injected would constitute about 18 per cent additional fluid which is not regarded as unduly diluting the blood or burdening the circulation.

As a rule the concentrations and doses of the different agents used represented those used in therapeutic practice, except in the case of non-therapeutic agents with which enough was used to produce effects. Before injection each solution was filtered through a quantitative filter (Whatman no. 40). The pollen extracts, phylacogen, typhobacterin and venarsen were used as such from the original containers. The solutions of arsphenamine and neoarsphenamine were prepared according to the directions accompanying each preparation.

Agar sol, agar sol-gel and toxified agar were prepared according to the directions of Novy (4) and collaborators. Agar sol is a 0.5 per cent suspension of agar in saline. This solidifies on cooling and must be melted before injection. When 1 part of agar sol is melted and diluted to 4 parts or 6 parts with normal saline, and is cooled in cracked ice for two hours, then thoroughly shaken, this constitutes agar sol gel 1 : 4 and 1 : 6, respectively. Toxified agar was prepared by heating 1 part of agar sol (0.5 per cent) on a water bath for two hours and adding to it 1 part of serum (rabbit's in our work) and incubating at 38°C., for fifteen minutes. The preparation is then ready for injection. Toxified acacia was prepared in the same way.

The acacia used in our work was the Turkey or Khardofan variety in small tears. The solutions were prepared by boiling for half an hour and then filtered in the usual way.

Practically all animals that survived the injections, or did not die shortly thereafter, were killed at the end of half an hour, some somewhat later, a few sooner. Autopsy was then performed, carefully examining the presence or absence of pulmonary distention, hemorrhages and congestion; the condition of the heart; coagulation of blood and absence or presence of visceral congestion.

Suitable sections of lungs were made and these were placed at once, while still fresh, into Zenker's fluid, for microscopic examination as to the absence or presence of bronchial distention, thrombi, conglutination and hemorrhages.

In the majority of experiments, 3 animals were injected with the different agents. All told 31 different agents were studied, 2 of which, acacia and agar, were injected in 3 and 4 different forms, respectively. In all 71 animals (70 guinea-pigs and one rabbit) were injected.

III. RESULTS AND DISCUSSIONS

The results with various thromboplastic agents, namely, the thromboplastins, coagulen and hemostatic serum have been described in a previous paper (8). The detailed results of the present series are presented in table 1 and summarized and classified as to principal phenomena produced in table 2. These may now be described.

1. Practically harmless agents

Out of the 31 injected only 2 agents were found to be practically harmless. Moderate degrees of pulmonary congestion occurred with mild ether anesthesia used in connection with skin incisions and with normal saline (2 out of 4 animals). In the particular experiment with ether here reported, the anesthetic was administered rather liberally, while with the remaining animals only a whiff of ether was used. The summary in table 2 indicates

TABLE 1

Effects produced by the intravenous injection of various colloids and other agents in guinea-pigs

NUMBER	WEIGHT OF GUINEA-PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Saline controls (0.9 per cent NaCl)							
	grams	*cc.*					
1		2		None	Killed, 20	Lungs collapsed	Normal
7	350	10		None	Killed, 86	Lungs collapsed	Normal
46	235	3		None	Killed, 48	Lungs partially inflated	Marked congestion
(0.85 per cent C. P. NaCl)							
52	220	3		None	Killed, 41	Lungs collapsed; pulmonary congestion; slight cardiac dilatation	Marked congestion, thrombosis of few large veins
Agar sol 0.5 per cent							
2	200	2	0.05 mgm.	Dyspnea; depression; trembling; death	Fatal, 12	Inflated lungs	
4	215	2	0.046 mgm.	Respiration stopped; relaxation of sphincters; death	Fatal, 5	Lungs inflated and hemorrhagic	Congestion, hemorrhage, thrombosis, edema
Agar sol gel (1 : 6)							
88	320	5	0.013 mgm.	Marked dyspnea and depression; convulsions; increased respiration; death	Killed, 31	Marked pulmonary distention and hemorrhages and slight congestion; moderate cardiac dilatation; moderate abdominal congestion. Blood clots rapidly	Marked distention, marked congestion, marked hemorrhage, thrombosis

Agar sol gel (1 : 4)							
19	200	3	0.015 mgm.	Marked dyspnea; spasms cyanosis; death	Fatal, 21	Marked pulmonary inflation; pale; no cardiac dilatation; no abdominal congestion	Marked distention, slight congestion, thrombosis
Agar gel (0.5 per cent agar 1 part and N. S. 1 part)							
13	200	2	0.025 mgm.	Dyspnea; jerky spasms	Killed, 35	Slight pulmonary inflation; hemorrhages; heart normal	Moderate distention, congestion, hemorrhage, edema, thrombosis
Toxified agar (1 part of 0.5 per cent agar and 4 parts of rabbit serum)							
3	200	2	0.01 mgm.	Dyspnea; air hunger; depression	Killed, 3 hrs., 9 m.	Marked pulmonary inflation	
6	205	2	0.01 mgm.	Increased. respiration spasms, dyspnea moderate	Killed, 3 hrs.	Lungs inflated	Congestion, hemorrhage, thrombosis
Acacaa (6 per cent and 0.5 per cent)							
5	200	2 cc. of 0.5 per cent	0.05 mgm.	Dyspnea; spasms; convulsions	Killed, 4 hrs., 12 m.	Lungs partially inflated	Congestion, conglutination thrombi
53	205•	3 cc. of 6 per cent	0.88 mgm.	Dyspnea; restlessness respiratory rate increased	Killed, 31	Lungs collapsed; no hemorrhages; no cardiac dilatation	Marked congestion, capillary conglutination thrombi
9	168	4 cc. of 0.5 per cent	0.12 mgm.	Dyspnea; trembling; depression	Killed, 38	Slight pulmonary inflation; hemorrhage; cardiac dilatation; liver dark	Congestion, conglutination thrombi

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA-PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCGPIC EXAMINATINN OF LUNGS
Acacia (6 per cent and 0.5 per cent)—*Continued*							
	grams	*cc.*					
14	260	3 cc. of 6 per cent	0.69 mgm.	Dyspnea; spasms; increased respiration	Killed, 35	Lungs inflated (partial); no cardiac dilatation	Marked distention, congestion, hemorrhage, edema, slight thrombosis
29	180	3 cc. of 6 per cent	1 mgm.	Marked dypsnea	Killed, 31	Lungs collapsed; fine punctate hemorrhages; slight cardiac dilatation; no abdominal congestion	Moderate distention, congestion, thrombosis
81	380	2.8 cc. of 6 per cent	0.44 mgm.	Increased respiration; restlessness; moderate dyspnea	Killed, 33	Marked pulmonary distention, hemorrhages and moderate congestion; moderate cardiac dilatation	Marked distention, marked congestion, marked hemorrhage
Toxified acacia (1 part of acacia 6 per cent and 2 parts of rabbit serum)							
27	175	3	0.34 mgm.	Marked dyspnea; respiration slowed, restlessness, depression	Killed, 32	Marked pulmonary inflation; hemorrhages; cardiac dilatation, no abdominal congestion	Marked distention, congestion, hemorrhage, thrombosis

Dialyzed acacia (6 per cent)							
45	360	3	0.5 mgm.	Dyspnea; respiration slowed, discomfort	Killed, 30	Marked pulmonary distention, congestion and minute hemorrhages; cardiac dilatation	Marked congestion, slight hemorrhage
Beef serum (whole; fresh)							
10	145	2	0.014 cc.	Respiration and heart stopped; death	Fatal, 2	Marked pulmonary inflation; hemorrhages marked; marked cardiac dilatation	Marked distention, congestion, hemorrhage, conglutination thrombi
Dog bile (whole; fresh)							
11	203	2	0.01 cc.	Marked depression; respiration slowed; death	Fatal, 6	Marked pulmonary inflation; hemorrhages; heart normal	Moderate distention, congestion, bile emboli and thrombosis
Gelatin (0.5 per cent and 6 per cent)							
153	500	3cc. of 6 per cent	0.35 mgm.	None	Killed, 35	Lungs collapsed and congested; cardiac dilatation	Marked congestion, capillary conglutination thrombi, only slight local distention
15	200	3 cc. of 0.5 per cent	0.075 mgm.	Slight dyspnea; jerky	Killed, 35	Partial or no pulmonary inflation; no cardiac dilatation, no abdominal congestion	Moderate distention, marked congestion, marked hemorrhage
44	260	3 cc. of 6 per cent	0.7 mgm.	Depression; dyspnea; respiration increased	Killed, 31	Slight pulmonary distention; marked congestion, some hemorrhages; marked cardiac dilatation	Congestion, hemorrhage

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA-PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNOS
Starch (2 per cent and 6 per cent)							
	grams	*cc.*					
154	450	3	0.4 mgm.	Dyspnea; increased respiration	Killed, 47	Lungs partially distended, markedly congested; moderate cardiac dilatation	Moderate general distention, slight congestion, marked hemorrhage
16	205	3 cc. of 2 per cent	0.29 mgm.	None	Killed, 32	Partial or no pulmonary inflation	Marked congestion, marked hemorrhage
						Hemorrhage upper right lobe; cardiac dilatation	Marked congestion, marked hemorrhage
46a	330	3 cc. of 6 per cent	0.54 mgm.	Convulsions; marked dyspnea	Killed, 30	Marked pulmonary inflation, hemorrhages and congestion; cardiac dilatation	
Collargol (1 per cent and 5 per cent)							
17	205	3 cc. of 5 per cent	0.73 mgm.	Respiration stopped; death	Fatal, 4	Slight pulmonary distention; blood granular; cardiac dilatation; abdominal congestion; blood granular	Marked congestion, marked thrombosis
30	175	2.8 cc. of 1 per cent	0.16 mgm.	Marked depression; respiration slowed, cyanosis, pupils dilated, facial twitching, death	Fatal, 30	Slight pulmonary distention; hemorrhage, blood less granular; cardiac dilatation; clot in heart	Moderate congestion, conglutination, thrombi

36	245	0.1 cc. of 2 per cent	0.0081 mgm.	Depression; dyspnea marked	Killed, 30	Lungs collapsed and congested; no abdominal congestion, heart slightly dilated	Marked distention, slight congestion, hemorrhage
Peptone (Witte; 5 per cent and 10 per cent)							
28	160	3 cc. of 5 per cent	0.94 mgm.	Dyspnea; convulsions; cyanosis; pupils dilated, relaxation of sphincters; death	Fatal, 7	Marked pulmonary inflation; lungs pale; no hemorrhages; marked cardiac dilatation; slight abdominal congestion	Marked distention
89	530	0.1 cc. of 10 per cent diluted to 3 cc. of N. S.	0.019 mgm.	Some dyspnea; increased respiration	Killed, 42	Marked pulmonary distention, congestion and hemorrhages; cardiac dilatation; no abdominal congestion	Marked distention, marked congestion, marked hemorrhage
Dextrin (6 per cent and 1 per cent)							
155	460	3	0.4 mgm.	Moderate dyspnea	Killed, 35	Lungs collapsed, congested and hemorrhagic; moderate cardiac dilatation; abdominal congestion	Marked congestion, no distention
25	185	3 cc. of 6 per cent	About 1 mgm.	Depression; dyspnea; respiration slowed	Killed, 40	Pulmonary inflation doubtful; marked hemorrhages	Marked distention, marked congestion, marked hemorrhage, edema

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA-PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Dextrin (6 per cent and 1 per cent)—*Continued*							
	grams	*cc.*					
87	410	3 cc. of 1 per cent	0.073	Dyspnea (?) restlessness	Killed, 33	Slight pulmonary distention, congestion and numerous hemorrhages, marked cardiac dilatation; moderate abdominal congestion	Marked distention, marked congestion, marked hemorrhage
Pancreatin (1 per cent)							
Rabbit	1 kgm.	5	0.053 gms. x kgm.	Shivering; spasms; increased respiration; dyspnea; depression	Killed, 2 hrs.	Distention of lungs; hemorrhages marked; abdominal congestion	
37	300	1.5 cc. of 1 per cent diluted to 3 cc. N. S.	0.05 mgm.	Marked dyspnea; restlessness; increased respiration	Killed, 37	Lungs inflated and hemorrhagic; no cardiac dilatation; no abdominal congestion	Moderate distention, marked congestion
Typhobacterin (Mulford)							
32	200	3	0.015 cc.	Jerky spasms; restlessness; dyspnea; ruffling of hair	Killed, 30	Marked inflation of both lungs; hemorrhage left lung, also congestion and pneumonia right lung; no abdominal congestion	Moderate congestion

			Pollen extracts (Mulford)				
31	245	2.7 cc. (ragweed)	0.011 cc.	Depressed; dyspnea marked	Killed, 30	Lungs collapsed and somewhat congested; heart somewhat dilated; no abdominal congestion	Marked congestion, slight hemorrhage
47	210	2.5 cc. (ragweed)	0.012 cc.	Marked depression, dyspnea; cyanosis; respiration increased	Killed, 33	Partial distention and congestion of lungs; left cardiac dilatation; no abdominal congestion	Marked congestion, slight hemorrhage
84	310	1 (Hay fever spring; mixed pollens)	0.000064 mgm. Pollen-nitrogen	Moderate dyspnea	Killed, 42	Marked pulmonary distention, hemorrhages and slight congestion; slight cardiac dilatation; moderate abdominal congestion	Moderate distention, marked congestion, slight hemorrhage, conglutination thrombi
85	530	1 (Hay fever fall; mixed pollens)	0.000037 mgm. Pollen-nitrogen	Increased respiration; dyspnea; restlessness	Killed, 40	Lungs collapsed; marked pulmonary congestion; no hemorrhage; marked cardiac dilatation; slight abdominal congestion	Moderate distention, marked congestion, slight hemorrhage, conglutination thrombi
96	440	1 (Hay fever fall; mixed pollens)	0.000022 mgm. Pollen-nitrogen	Dyspnea; marked increase in respiration	Killed, 48	Pulmonary distention, marked congestion and multiple hemorrhages; marked cardiac dilatation; moderate abdominal congestion	Marked congestion

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA-PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Arsphenamine (0.5 per cent)							
	grams	*cc.*					
35	200	0.3 cc. in 3 cc. N. S. (D. R. L.)*	0.0075 mgm.	Marked dyspnea; respiration slowed; depression; hair ruffled	Killed, 32	Lungs collapsed and markedly congested; cardiac dilatation both sides, no abdominal congestion	Marked congestion, slight hemorrhage
34	235	0.33 cc. (D. R. L.)	0.007 mgm.	Dyspnea; hair ruffled; jerky; shivering	Killed, 42	Lungs collapsed and congested; cardiac dilatation; no abdominal congestion	Marked distention, marked congestion, marked hemorrhage
105	400	0.7 cc. (T. L.)†	0.009 mgm.	Marked dyspnea; choking; restlessness	Killed, 41	Lungs collapsed, and congested with few small hemorrhages, slight cardiac dilatation and moderate abdominal congestion. Blood clots in 2 minutes	Marked distention, marked congestion, marked hemorrhage, conglutination thrombi
Glycogen (0.5 per cent)							
38	350	3.5	0.05 mgm.	Depression; dyspnea; spasms; restlessness	Killed, 37	Marked pulmonary distention and congestion; pneumonia right lung; cardiac dilatation; no abdominal congestion	Slight distention, moderate congestion

86	230	3	0.065 mgm.	Dyspnea; sick	Killed, 30	Pulmonary distention and congestion; marked cardiac dilatation and abdominal congestion	Slight distention, marked congestion
Nuclein solution (Abbott; about 0.02 per cent)							
40	200	1.4 cc. diluted to 3 cc. N. S. (old)	0.0014 mgm.	Depression; respiration slow and shallow; no dyspnea	Killed, 34	Very slight distention of lungs; considerable congestion; no cardiac dilatation; no abdominal congestion	Marked congestion
104	330	0.5 cc. (fresh)	0.0015 cc. or 0.00033 mgm.	Marked increase in respiration; restlessness; no dyspnea	Killed, 45	Slight pulmonary distention, marked hemorrhages and moderate congestion; moderate cardiac dilatation, and abdominal congestion	Moderate congestion, slight hemorrhage
115	430	3 (fresh)	0.009 cc. or 0.0032 mgm.	Increased respiration; no dyspnea	Killed, 31	Lungs markedly distended, congested and hemorrhagic; moderate cardiac dilatation and abdominal congestion; blood clotted in 2 minutes	Marked distention, marked congestion, marked hemorrhage

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA-PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
				5 per cent Inulin (1:10 N. S.; suspension)			
	grams	*cc.*					
39	210	2.1 cc. diluted to 3 cc.	0.05 mgm.	Marked dyspnea; respiration shallow; cyanosis; restless	Killed, 30	Lungs collapsed and congested; cardiac dilatation; no abdominal congestion	Marked congestion, slight hemorrhage
83	370	3	0.81 mgm.	Marked dyspnea; increased respiration; restlessness	Killed, 43	Lungs collapsed and congested; no hemorrhages; marked cardiac dilatation	Moderate congestion
				Neoarsphenamine (0.6 per cent)			
43	220	0.55 cc. to 3 cc. N. S. (S. D. Co.)‡	0.015 mgm.	Increased respiration; marked dyspnea; spasms	Killed, 31	Lungs collapsed; marked congestion; dilatation right heart; no abdominal congestion	Moderate congestion, hemorrhage
93	260	0.65	0.015 mgm.	Dyspnea; increased respiration	Killed, 38	Pulmonary distention; congestion and hemorrhages; slight cardiac dilatation; slight abdominal congestion	Moderate distention, marked congestion, marked hemorrhage
108	440	1.1 cc. (T. L.)†	0.015 mgm.	None	Killed, 30	Lungs slightly distended, congested and hemorrhagic; moderate cardiac dilatation and abdominal congestion	Marked congestion

			Sodium arsenate (0.6 per cent)				
42	285	0.40 cc. to 3 cc. N. S.	0.0084 mgm.	Depression; respiration slowed; dyspnea; cyanosis restless	Killed, 32	Slight pulmonary distention and congestion; slight cardiac dilatation, slight abdominal congestion	Moderate distention, moderate congestion, hemorrhage
103	350	0.22 cc. to 3 cc. N. S.	0.0038 mgm.	Increased respiration; hair ruffled	Killed, 33	Lungs collapsed and congested; marked cardiac dilatation; very slight abdominal congestion	Moderate distention, marked congestion, marked hemorrhage, thrombosis, edema
			Rabbit's serum (dialyzed)				
47	330	3	0.0091 mgm.	Dyspnea; restlessness; respiration increased	Killed, 48	Slight pulmonary distention and marked congestion; heart dilated; slight abdominal congestion	Marked congestion, slight hemorrhage
			Ether and operation (control)				
58	280	No intravenous injection		None	Killed, 37	Lungs collapsed; distinct congestion; no cardiac dilatation; slight abdominal congestion	Marked congestion, slight hemorrhage
			Human serum (pooled)				
62	190	3	0.016 cc.	Dyspnea; convulsions; cyanosis; depression	Killed, 34	Marked pulmonary distention; hemorrhages and edema in lungs; slight cardiac dilatation; no abdominal congestion	Moderate distention, marked congestion, thrombosis

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Congo red (1 per cent)							
	grams	*cc.*					
80	400	3	0.075 mgm.	Increased respiration; dyspnea	Killed, 44	Marked pulmonary distention, hemorrhages and congestion; marked cardiac dilatation; moderate abdominal congestion	Marked distention, marked congestion, hemorrhage
90	220	1	0.045 mgm.	Increased respiration; slight dyspnea	Killed, 40	Slight pulmonary distention, considerable congestion, no hemorrhages, cardiac dilatation; abdominal congestion	Marked congestion
151	460	3	0.065 mgm.	Marked depression; dyspnea twitching; death	Fatal, 23	Lungs collapsed and moderately congested, marked cardiac dilatation and abdominal congestion	Marked congestion. slight local distention
Althea (15 per cent extract)							
82	300	3	1.5 mgm.	Some increase in respiration	Killed, 35	Very slight distention of lung; marked congestion and slight hemorrhages in lungs; slight cardiac dilatation; marked abdominal congestion	Marked congestion, conglutination thrombi

109	440	3	1.02 mgm.	Increased respiration; dyspnea	Killed, 36	Lungs markedly distended, congested and hemorrhagic; marked cardiac dilatation and abdominal congestion; blood clots in 3 minutes	Marked congestion
152	420	3	1.07 mgm.	Marked increase in respiration; twitching of ears	Killed, 33	Lungs congested markedly and collapsed; cardiac dilatation; moderate abdominal congestion	Moderate congestion, few platelet thrombi, no distention
Venarsen (sodium cacodylate)							
95	220	0.07 diluted to 0.5 cc. with saline (I.Co) §		None	Killed, 44	Lungs collapsed and slightly congested, very slight abdominal congestion	Marked congestion, slight hemorrhage
Phylacogen (mixed infection; Parke Davis Company)							
94	260	1	0.0035 cc.	Restlessness; shivering; increased respiration	Killed, 36	Slight pulmonary distention; marked congestion and minute hemorrhages; slight cardiac dilatation and abdominal congestion	Slight distention, marked congestion, slight hemorrhage, slight conglutination thrombosis

TABLE 1—*Concluded*

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM	PRINCIPAL SYMPTOMS	FATE OF ANIMAL (END OF MINUTES)	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Phylacogen (mixed infection; Parke Davis Company)—*Continued*							
	grams	*cc.*					
102	500	1	0.002 cc.	Increased respiration	Killed, 37	Lungs collapsed; few minute hemorrhages, considerable congestion, moderate cardiac dilatation and abdominal congestion	Marked congestion, slight hemorrhage
110	380	2	0.0053 cc.	Very marked dyspnea; increased respiration; restlessness; shivering	Killed, 34	Very little pulmonary distention, slight congestion and hemorrhages; slight cardiac dilatation; blood clots in 2 minutes	Marked distention, marked congestion, marked hemorrhage

* D.R.L. = Dermatological Research Laboratories.
† T.L. = Takamine Laboratory Inc.
‡ S.D.Co. = Synthetic Drug Co.
§ I.Co. = Intravenous Products Co.
N.S. = Normal saline.

TABLE 2

Classification of agents as to injury to the circulation and respiration in the majority of animals

INJURY	CRITERIA	AGENTS AND REMARKS
Practically harmless	Anaphylactoid symptoms absent Pulmonary distention absent macroscopically and microscopically Pulmonary congestion present in some, hemorrhage absent in all Pulmonary thrombi absent Cardiac dilatation absent or unimportant	Ether anesthesia and operation alone (pulmonary congestion present) Normal saline (0.9 per cent NaCl)
Respiratory system chiefly	Anaphylactoid symptoms present Pulmonary distention present macroscopically and microscopically Pulmonary congestion and hemorrhage present Pulmonary thrombi generally present Cardiac dilatation absent or doubtful	Agar sol (fatal) Agar gel Agar sol-gel (fatal) Agar toxified with rabbit's serum Bile (dog's; fatal) Human serum (thrombi absent) Typhobacterin (thrombi and congestion absent)
Circulatory system chiefly	Anaphylactoid symptoms usually present Pulmonary distention absent macroscopically and microscopically Pulmonary congestion and hemorrhage present Pulmonary thrombi generally absent Cardiac dilatation present	Althea extract (thrombi in 2 out of 3 animals) Arsphenamine (microscopic distension; thrombi in 1 out of 3 animals) Collargol (fatal; microscopic distention and thrombi present in 2 out of 3 animals) Congo red (fatal; microscopic distention in 1 out of 3 animals) Dextrin (microscopic distention) Gelatin (symptoms absent in 1 animal; conglutination present in 1 animal) Inulin

TABLE 2—*Concluded*

INJURY	CRITERIA	AGENTS AND REMARKS
Circulatory system chiefly	Anaphylactoid symptoms usually present Pulmonary distention absent macroscopically and microscopically Pulmonary congestion and hemorrhage present Pulmonary thrombi generally absent Cardiac dilatation present	Neoarsphenamine (microscopic distention in 1 out of 3 animals) Phylacogen (microscopic distention in 2 out of 3 and thrombi in 1 out of 3 animals) Rabbit serum Sodium arsenate (symptoms absent in 1 and thrombi present in 1 out of 2 animals) Venarsen (symptoms absent)
Circulatory and respiratory systems	Anaphylactoid symptoms present Pulmonary distention present macroscopically and usually microscopically Pulmonary congestion and hemorrhage generally present Pulmonary thrombi present or absent Cardiac dilatation present, usually also abdominal congestion	Acacia, 50 per cent, 6 per cent and less Acacia, dialyzed (no microscopic distention) Acacia toxified with rabbit's serum (no microscopic distention) Beef serum (fatal; thrombi present) Glycogen (microscopic distention and thrombi absent) Nuclein solution (no thrombi) Pancreatin (thrombi absent) Peptone (Witte; fatal; thrombi absent) Pollen extracts (thrombi and microscopic distention in 2 out of 5 animals) Starch (microscopic distention in 1 out of 3 animals and thrombi absent)

that pulmonary congestion was uniformly present in all the experiments, indicating that in part, at least, the ether anesthesia was responsible. However, it is seen that in the experiments with other agents except normal saline, hemorrhage was a rather constant accompaniment of pulmonary congestion, indicating

that these agents per se probably accentuated the congestion as a result of which hemorrhage was the natural sequel. Therefore, the effects of the ether anesthesia can be largely discounted. This is further supported by the absence of any demonstrable pulmonary and other changes whatsoever in the majority of animals injected with normal saline, also anesthesized with ether.

Conclusions. Mild ether anesthesia and the intravenous injection of saline are practically harmless to the circulatory and respiratory systems of guinea-pigs, but the remaining 29 of the 31 agents studied were found to be distinctly harmful in varying degrees.

2. Agents whose injurious effects are expressed through the respiratory system chiefly

Agar. Of all the colloids that were tested agar was found to be the most injurious, and this was expressed principally through the respiratory system. As a rule, thrombi and conglutination of corpuscles were observed in the pulmonary capillaries. Aside from this, nothing abnormal in the circulation was observed. In a future communication it will be shown that agglutination of corpuscles by agar can also be reproduced in vitro, which, when taken together with thrombus formation in vivo, indicates that the primary mechanism of agar disturbances is associated with or due to its effect on the red blood corpuscles. This mechanism appears to be associated with the peculiar nature of agar, but not necessarily because of its colloidal nature. Somewhat different symptoms and effects were observed with other colloids, such as acacia, dextrin and starch. Agar appears to stand quite alone in the very prompt and striking pulmonary effects which it produces when injected intravenously.

The quantity of agar necessary to produce these profound effects and even death, is remarkably small, namely, about 0.014 to 0.05 mgm. per gram of body weight or about 0.014 to 0.025 per cent concentration of agar in the blood of a 200 to 300-gram animal in our experiments. It was shown previously by Novy that the smaller the aggregates, such as are presumably obtained

in agar sol-gel, the more profound the effects. In our experiments no special differences between agar sol, agar gel, and agar sol-gel were observed, and toxifying agar with rabbit's serum did not increase its toxicity in the 2 experiments that were performed. Fatalities occurred with the agar sol and agar sol-gel. For our purposes it sufficed to show that agar as an example of a typical colloid is definitely injurious in small quantities, also that this injury produced anaphylactoid phenomena, which outwardly, at least, that is, in a clinical way, resembled the alleged disturbances produced by the intravenous injection of arsphenamine and other agents, if not anaphylactic shock itself.

So far as the results of the injection experiments here reported are concerned, the marked inflation of the lungs produced could not be differentiated from the marked inflation of anaphylactic shock. In anticipation of results to be reported in subsequent papers it can be stated that the pulmonary inflation is not due to stimulation of smooth muscle by the agar. This was determined by perfusion of lungs, the treatment of both intact and surviving lungs with atropine, epinephrine and papaverine (to produce bronchial relaxation) and experiments with surviving intestine and uterus. The perfused lungs distended rapidly and there was a stoppage of the perfusion flow promptly. This would indicate peripheral stimulation of bronchial muscle. However, in the absence of relief by atropine and papaverine of this effect, and the absence of uterine and intestinal stimulation, it is concluded that agar does not stimulate smooth muscle. There is no reason to believe that bronchial muscle is specifically stimulated by agar, or that agar is more powerful than histamine. In addition, the pulmonary vessels (arterioles and capillaries) of intact animals injected with agar sol gel contained numerous thrombi, emboli and conglutinated corpuscles. The vessels of lungs perfused with agar sol-gel were loaded with agar emboli and thrombi and this caused the perfusion fluid to stop.

On the other hand, no emboli, thrombi or conglutinated corpuscles were demonstrable in perfused or intact lungs of anaphylactic shock, after peptone, certain serums and histamine. All of these agents were also active smooth muscle stimulants in

other regions besides bronchi, namely, uterus and intestine. Pulmonary inflation occurred and this was relieved in intact lungs (except histamine) by atropine and epinephrine and in perfused lungs by atropine and papaverine. It is well known that epinephrine and atropine can relieve the bronchoconstriction of perfused lungs due to a variety of stimulants, but this is not true of agar. It is the occurrence of pulmonary emboli and thrombi with agar, resulting in passive bronchoconstriction (really compression of bronchi), that, we believe, is of the greatest significance in explaining the pulmonary distention and resultant phenomena. This at once separates the action of agar from true anaphylactic shock, and places all similarly acting agents into the same category with agar. It is deemed necessary to make this plain now, since this will facilitate and simplify the discussion of the remaining agents in this paper. That is, either an agent will act like agar producing passive bronchoconstriction and pulmonary inflation with the attendant symptoms of asphyxia, etc., or act partially like agar in which the effects are mixed with those of asphyxia, occurring primarily or secondarily, depending on the extent of damage to the circulation. A priori, therefore, it is obvious that the disturbances arising from the intravenous injection of various agents (arsphenamine, acacia, etc.) may have been erroneously attributed to anaphylaxis and anaphylactic shock arising therefrom. This will be substantiated more fully in the discussions to follow and finally also confirmed in various ways in future publications.

Bile. The single experiment performed with dog's bile indicates that it is distinctly injurious, producing a strong pulmonary inflation, thrombi and death, behaving in this respect not unlike agar. Of what importance this might be for the elucidation of the symptoms of jaundice is not known.

Human serum. The results obtained were similar to those commonly obtained with other toxic foreign serums such as beef serum previously studied by one of us (9). The general absence of thrombi and the well known augmentor effects on smooth muscle suggest a different mechanism of action from agar.

Typhobacterin. The results obtained in the one experiment that was performed agree entirely with the effects usually obtained after the injection of dead organisms, vaccines and similar preparations. The injection of larger organisms such as dead trypanosomes produces similar effects on intravenous injection (Novy). Whether the mechanism of action is different from, or similar to agar action as believed by Novy, can not be said at this time, since the subject could not be adequately investigated by us.

Conclusions. The injury produced by the intravenous injection of the following agents is expressed through the respiratory system chiefly, producing definite anaphylactoid symptoms, and pulmonary distention, congestion, hemorrhages and thrombi without definite cardiac dilatation; agar sol (0.5 per cent), agar gel (0.5 per cent), agar sol-gel (1 : 4 and 1 : 6), dog's bile, human serum and typhobacterin. Pending further proof the mechanism of agar action may be stated to consist of passive bronchoconstriction with consequent phenomena of asphyxia. Consequently the disturbances of intravenous administration following the injection of various agents in unsensitized animals do not necessarily constitute true anaphylaxis or anaphylactic shock.

3. Agents whose injurious effects are expressed mainly through the circulatory system

Althea (marshmallow). According to Ono (10) althea is a strong agglutinator of corpuscles in vitro. In our injection experiments, fine pulmonary thrombi after the intravenous injection of althea extract (15 per cent) were present in 2 out of 3 animals. Extracts of althea, even when fresh, are acid in reaction. This may explain the results in vitro, but in vivo the effects would depend on the quantity injected and available reserve alkali for neutralization. The dosage used was rather large and althea is also colloidal in nature. It is seen that pulmonary distention was generally absent although symptoms were present, due no doubt to the injury to the circulation as indicated by cardiac dilatation, pulmonary congestion and hemorrhages which were present.

Arsphenamine. It is claimed by Stokes (11) that arsphenamine is precipitated from its colloidal form in the circulation, and this or the precipitation of blood colloids evokes the "nitritoid crises" of arsphenamine poisoning which is a form of anaphylactic shock. The term "nitritoid crises" was introduced by Milian (12) because of the similarity of the symptoms to those from amyl nitrite. These consist principally of redness of face, dyspnea, feeling of anguish and distress, cough and precordial pain in human individuals after intravenous injection (Berman (13); Beeson (14); Hirano (15)). Berman explains the arsphenamine disturbances by precipitation occurring with sera of individuals exhibiting so-called "nitritoid crises." Although Berman claims to obtain precipitation in vitro with the sera of these individuals and not with those without symptoms, the precipitation of arsphenamine in the blood stream still remains to be demonstrated. We have observed frequently that insufficiently alkalinized solutions of arsphenamine will precipitate on the addition of 0.9 per cent NaCl, but this does not occur if a slight excess of alkali is added. From their experimental work on dogs, Jackson and Smith concluded that arsphenamine raises the pulmonary arterial pressure and dilates the right heart and this seemed to be associated with the alkalinity of arsphenamine. Pulmonary capillary obstruction such as by thrombi, emboli, etc., was not looked for by Jackson and Smith (16). The anaphylactic explanation invoked by Stokes does not appear plausible in view of Auer's (17) work with arsphenamine on guinea-pigs. Hirano (18) claims a diminution in epinephrine content of the adrenals is responsible for the symptoms.

In the experiments performed by us with therapeutic and even smaller doses of alkalinized arsphenamine prepared in the usual way, the guinea-pigs showed no gross pulmonary distention, and only microscopic distention in 2 out of the 3 animals. Conglutination thrombi were present in one animal. Congestion and hemorrhages in the lungs were marked in all animals. There was also definite cardiac dilatation. Taking all of the phenomena into consideration, together with our experience with other agents on about 160 animals, the results with the 3 animals injected in-

dicate that arsphenamine, even in very small doses, injures the circulation giving rise to symptoms of an anaphylactoid nature. The perfusion experiments to be reported later also showed no pulmonary distention, indicating definitely that the arsphenamine reactions are not like anaphylaxis or anaphylactic shock. When precipitated solutions are injected, the disturbances might be like those from agar, and due to pulmonary emboli and thrombi. This would not necessarily occur because of the alleged colloidal form of arsphenamine.

This is supported by the results of our experiments with sodium arsenate, which is a crystalloid and produced practically the same phenomena as arsphenamine and neoarsphenamine. There is no reason to believe that sodium arsenate is transformed to a colloidal form in the circulation.

Collargol. This is a silver-protein suspensoid colloid recommended for intravenous injection as an antiseptic and is claimed to be harmless. Different doses in different concentrations (within the therapeutic range in 2 animals) were injected into 3 guinea-pigs. All were distinctly harmed as indicated by the presence of definite symptoms. Two injections were fatal. These were the higher doses used. Two of the 3 animals showed the presence of pulmonary thrombi; absent or slight distention, congestion of variable degree and hemorrhage in only one animal. Definite cardiac dilatation was present in all. Collargol, therefore, is distinctly injurious and principally to the circulatory system, the claims of its advocates as to harmlessness to the contrary notwithstanding.

Congo red. This is another suspensoid colloid, differing from collargol chemically, but resembling it pharmacologically. The data in table 1 indicate that congo red in small quantities is a distinctly harmful agent. Definite symptoms of respiratory distress were produced together with definite pulmonary distention in 1, and slight distention in another out of the 3 animals injected; marked congestion in all and hemorrhage in one. Cardiac dilatation was uniformly present. Apparently, therefore, congo red injured chiefly the circulation, in the absence of pulmonary thrombi and emboli.

Dextrin. Gross distention of the lungs after injection of dextrin was practically absent, but present microscopically in 2 out of the 3 animals treated. Marked pulmonary congestion was present in all and hemorrhage in 2 animals. This together with the cardiac dilatation indicates principal injury to the circulatory system which is quite sufficient to explain the symptoms of respiratory distress that were present.

Gelatin. Gelatin has been used to some extent as a constituent of colloidal solutions intravenously for support of the circulation in shock, and in the treatment of hemorrhage. The objections to its use have been mainly the dangers of infection from imperfect sterilization. Other objections might be raised on the basis of results obtained by us with guinea-pigs.

The changes produced were not uniform. Symptoms of respiratory distress were present in the majority of animals; also moderate pulmonary distention microscopically, marked hemorrhages, conglutination thrombi (one animal) and definite cardiac dilatation. On the whole, it appears that the injury produced by gelatin is of circulatory origin, the pulmonary distension being rather variable and uncertain and perhaps depending on asphyxia as a result of the circulatory injury. It must be concluded that solutions of gelatin are not devoid of harmful effects when injected intravenously.

Inulin. Inulin has been used by Nathan (2) and DeKruif and German (19) for the production of artificial anaphylatoxin, and apparently it is more effective when toxified with serum. In our experiments inulin alone in solution was used. Marked symptoms of respiratory distress were produced together with pulmonary congestion, but no distention. Thrombi were absent, and the heart was definitely dilated. The symptoms, therefore, appear to be associated with circulatory injury.

Phylacogen (Parke Davis Company). The preparation called "Mixed Infection" was injected into 3 animals. It is highly recommended and advocated by the manufacturers for intravenous use in the treatment of different kinds of infections, even of doubtful etiology. The results obtained by us do not at all agree with the claims of harmlessness by the manufacturers.

No definite gross pulmonary distension was observed, but in 2 of the animals this was found to exist microscopically in marked and slight degrees. The presence of marked pulmonary congestion and hemorrhages was uniform throughout, and in one animal there was conglutination thrombosis. The symptoms of respiratory distress, which were present in all animals, appear to be principally of circulatory origin. It is obvious that phylacogen is distinctly injurious when used intravenously and might do more harm than good as an alleged therapeutic agent. This agrees with well founded suspicions of and clinical reports concerning phylacogens as not only empirical but actually dangerous remedies.

Neoarsphenamine. The effects of this arsenical (from 3 different sources) in therapeutic doses were similar to those of arsphenamine. The injury appears to be of circulatory origin as indicated by the uniform cardiac dilatation, marked pulmonary congestion and hemorrhages without definite gross or practically any microscopic distention. This is as would be expected with an arsenical agent.

Rabbit's serum. In the one experiment that was performed definite symptoms of respiratory distress were present, together with marked pulmonary congestion and slight hemorrhages, but no distention and thrombi. The heart was dilated, indicating that the serum was definitely harmful to the circulatory system. This serum, which was used to prepare toxified agar and acacia according to Novy's method, could account for a portion at least of the effects obtained with these preparations. The results with serum alone agree with the usual effects.

Sodium arsenate. This crystalloid arsenical was used as a control for the results obtained with arsphenamine, which is claimed by Danysz (7) to exist in a colloidal state in the circulation. However, the results being practically the same as with arsphenamine, and also neoarsphenamine, there is no reason to invoke any fancied peculiarity with arsphenamine. The effects of all the arsenicals tried are, no doubt, due to the arsenic though perhaps modified by the different forms in which the arsenic existed. Sorev (20) found that the primary mechanism of death in

rabbits after the intravenous injection of colloid and crystalloid arsenicals was the same, namely, obstruction of pulmonary capillaries with the usual symptoms of asphyxia. Launoy (21) concluded that death from colloidal and other arsenical compounds occurs in about the same way. Our results justify the conclusion that the symptoms after the injection of arsenical compounds, whatever the form of arsenic, are of circulatory origin.

Venarsen. Sodium cacodylate is the principal constituent of this arsenical preparation. In the one experiment that was made, symptoms were absent, lungs collapsed, and there was marked pulmonary congestion with hemorrhage. Apparently, venarsen acts like other arsenicals, injuring the circulation principally.

Conclusions. The following agents when injected intravenously were found to produce definite symptoms of respiratory distress together with pulmonary congestion and hemorrhages but pulmonary distension and thrombi, as a rule, were absent; althea extract, arsphenamine, collargol, congo red, dextrin, gelatin, inulin, neoarsphenamine, phylacogen (mixed infection), rabbit's serum, sodium arsenate and venarsen. The injuries produced appear to be principally concerned with the circulatory system. The intravenous administration for therapeutic purposes of such agents as collargol and phylacogen, which still lack a scientific basis, is unjustified.

4. Agents whose injurious effects are expressed through the circulatory and respiratory systems

Acacia. The results with acacia, dialyzed acacia and acacia toxified with rabbit's serum were practically the same. The preparation and dosage of acacia were the same as is used therapeutically in the treatment of shock (traumatic, from hemorrhage, etc.). Symptoms of respiratory distress were present in all of the 8 animals injected, and pulmonary distention was definite macroscopically and microscopically in the majority. Marked congestion of the lungs was present in all, and marked hemorrhages in 5 out of the 8 animals. Thrombi in the pulmo-

nary vessels were present in 6 out of the 8 animals. Cardiac dilatation of variable degrees was present in all of the animals. The results obtained prove conclusively that acacia, when injected in doses and concentrations corresponding to those used in the treatment of traumatic shock, is definitely injurious. This is contrary to the results, pertaining chiefly to symptoms, reported by De-Kruif (22), but confirmative of the conclusions reached by Kruse (23), and opinions held by many surgeons with extensive experiences in the treatment of shock during the recent war. In a later paper it will be shown that concentrations of acacia corresponding to those occurring in human blood agglutinate red blood corpuscles in vitro. This agrees with the property of acacia to form thrombi in vivo.

Beef serum. Except for the presence of pulmonary thrombi, the results obtained with the single animal injected are confirmative of those previously obtained by one of us (9) and others, namely, that the intravenous injection of beef serum in guinea-pigs is distinctly injurious, involving both the respiratory and circulatory systems. The results as to thrombi would probably be different with a greater number of animals.

Glycogen. When injected intravenously in the concentration of 0.5 per cent, glycogen is distinctly injurious. This is indicated by the presence of symptoms of respiratory distress, marked gross pulmonary distention and congestion and cardiac dilatation. Thrombi were not found after this colloid. The results of perfusion experiments to be reported later indicate no definite bronchoconstriction or augmentor action for other smooth muscle. The injury, therefore, appears to be mainly circulatory, the respiratory disturbance arising secondarily as a result of the circulatory injury. It is obvious why glycogen should not reach the circulation as such before conversion to the simpler carbohydrates.

Nuclein solution.[2] This is an aqueous extract of the wheat germ recommended for intravenous and hypodermic administration. Three animals were injected with doses ranging from

[2] Supplied by the Abbott Laboratories, Chicago.

0.0015 to 0.009 cc. per gram of body weight. The respiratory rate was increased in the majority although no dyspnea was noticeable. Variable degrees of pulmonary distention, congestion and hemorrhages, and moderate cardiac dilatation were present in all. Pulmonary thrombi were absent. The results indicate that nuclein solution in the dosage used is distinctly injurious when injected intravenously.

Pancreatin. With this a guinea-pig and a rabbit were used. The results obtained resemble those following the intravenous injection of peptone in guinea-pigs, as would be expected. Hemorrhages in the abdominal and pulmonary viscera occurred as claimed by Wago (24).

Peptone. The experiments with peptone, a protein colloid, served as controls in the same way as those with the different foreign serums for the non-protein colloids and other agents that were tested. The 2 animals that were injected responded in the usual way to peptone. The larger dose was promptly fatal, producing typical symptoms of respiratory distress, marked pulmonary inflation, and delayed coagulation of the blood as in anaphylactic shock. The smaller dose (0.019 mgm. per gram) which corresponded to that recommended by Nolf (25) for the treatment of certain infectious and febrile conditions in human individuals produced essentially the same effects as the larger dose, except death, and the vascular effects as indicated by marked pulmonary congestion and hemorrhages were more marked.

The heroic effects, which small doses of peptone are known to produce in human individuals, and the fact that they are distinctly injurious and that larger doses may be fatal should enjoin caution in the promiscuous and careless application of this kind of therapy. It is interesting to note that pulmonary thrombi are absent, as a rule, after the injection of peptone, foreign serums and in truly anaphylactic animals, indicating that peptone, foreign serums and histamine produce their effects in a different way from the various non-protein colloids and similar agents reported in this paper.

Pollen extracts.[3] These were tested because of their plant protein nature and their common use in the treatment of hay fever. While they are not recommended for therapeutic use intravenously, it is conceivable that enough of these may be absorbed when administered hypodermically or applied on the mucous surfaces. Five animals were injected with extracts prepared from ragweed, mixtures of grasses used for spring hay fever and mixtures used for fall hay fever. The results with the different extracts were about the same.

Symptoms of respiratory distress as a rule were present, but gross pulmonary distension was variable, and microscopically occurred only in 2 out of the 5 animals. Marked pulmonary congestion was uniform throughout; hemorrhages occurred in the majority (4) and conglutination thrombi in 2 animals. The heart was generally dilated. These results indicate injury to the circulatory system principally, the symptoms of respiratory disturbance occurring secondarily as a result of the circulatory injury, and perhaps of asphyxial origin. The conclusion that pollen extracts when injected into (or reaching) the circulation are distinctly injurious is justified. Whether any such quantities could reach the circulation as were used in our experiments and when administered by routes other than intravenous is not known.

Starch. Merck's soluble starch (2 per cent and 6 per cent) was injected into 3 animals. All were definitely and markedly injured as indicated by the presence of symptoms of respiratory distress, moderate distention of the lungs together with marked pulmonary hemorrhages and definite congestion and definite cardiac dilatation. The solutions in each case were filtered and resembled in appearance those of acacia. However, no pulmonary thrombi were observed. Okasaki (26) reports the presence of starch granules in the pulmonary capillaries after intravenous injection in rabbits. Our results are sufficient to indicate that such a colloid as starch, non-protein in character and otherwise harmless, when injected intravenously is definitely harmful, in-

[3] Supplied by H. K. Mulford Company, Philadelphia.

juring the circulatory system principally, the symptoms of respiratory distress occurring secondarily.

Conclusions. The intravenous injection of the following agents injures the circulatory and respiratory systems as indicated by the presence of anaphylactoid symptoms, pulmonary distention, congestion and hemorrhage together with cardiac dilatation: Acacia, plain dialyzed and toxified with rabbit's serum, beef serum, glycogen, pancreatin, peptone (large and very small doses), pollen extracts and starch. Thrombi in pulmonary vessels occurred after the injection of acacia in therapeutic doses and concentrations, and pollen extracts. The indiscriminate and careless administration of acacia and peptone intravenously should be enjoined with caution.

5. *Dangers of intravenous medication*

The results of the experiments in this investigation are sufficiently conclusive against the promiscuous, careless and unwarranted use of the intravenous method of administering drugs as a routine measure for therapeutic purposes. The disadvantages of damage to important systems like the circulation and respiration certainly outweigh the advantages that are to be gained from the administration of remedies of doubtful efficacy and those whose actions are produced equally or about as readily by other methods. Notable exceptions to this are such well known and tried remedies as strophanthin and arsphenamine.

The dangerousness of certain remedies can be sufficiently predicted from their pharmacological action and toxicity. On the other hand such colloidal agents as agar, acacia, gelatin, glycogen, starch and dextrin which possess no demonstrable pharmacological actions and are non-toxic in the usual sense of the word, that is, because of their inherent chemical properties, and are, in fact, practically inert, yet when injected intravenously into animals are distinctly injurious. This certainly could not be predicted from our knowledge of their chemical and pharmacological properties; and emphasizes the fact that without actual trial, speculation may lead to erroneous conclusions. This

should be impressed with an agent like acacia, which, although already suspected of deleterious qualities in clinical practice, was nevertheless advocated with considerable zeal. How much actually can be gained by the intravenous injection of acacia in shock, and similar conditions, in view of its harmful qualities, granting even that its use is dictated by certain clinical experience or is well established on physiological grounds, may indeed be doubted. Its use on a physiological basis is doubted by Prof. G. N. Stewart (27).

What has been said of acacia, also pertains to peptone, which is being advocated as a therapeutic measure. With proteins the deleterious effects are better appreciated. With certain other agents which partake of both the colloidal and protein nature, such for instance as collargol, the possibilities of harm should be even more patent. This is illustrated by the action of congo red, which is a suspensoid colloid similar in type physically to the silver portion of collargol, though non-protein in nature. Further illustrations are found in the various hydrophilic colloids discussed above.

Concerning the remaining agents, which are used or advocated for use therapeutically, namely, arsphenamine, neoarsphenamine, venarsen, phylacogen, nuclein solution and typhobacterin, perhaps enough has been said in the text. The unscientific basis for the use of phylacogens and even nuclein has been sufficiently pointed out. The intravenous injection of phylacogen, at least, in large or small doses is decidedly unjustifiable. Typhobacterin and other vaccines are used hypodermically in their proper rôle, and the extensive successful use of typhoid and some other vaccines testifies adequately to their relative harmlessness when used in this way. The heroic effects occurring after the intravenous injection of typhoid vaccine and foreign proteins as practiced by some in the treatment of rheumatic fever, etc., does not appear so well justified.

Concerning the organic arsenicals, like arsphenamine, neoarsphenamine and venarsen, the results obtained were about the same as with sodium arsenate, an inorganic arsenical. The toxicity of these agents is generally admitted, and our experiments

indicate that even in very small doses the action of arsenic on the circulatory system is still evident. The invocations of colloidal and other transformations for arsphenamine in the circulation to explain the so-called "nitritoid crises" appear to be subtle distinctions that are unnecessary. That these disturbances appear to be concerned to a considerable extent with the method of preparing arsphenamine solutions (insufficient alkali, precipitation, etc.) is quite evident from the detailed and careful instructions for this by the manufacturers of these preparations and in part, at least, to the arsenic, but not to true anaphylaxis or anaphylactic shock as indicated by the results of our experiments and also of others. This brings us to the consideration of the relation of our results to anaphylaxis.

Conclusion. The results obtained are decidedly against the promiscuous and unwarranted use of the intravenous method of administering drugs as a routine therapeutic measure, particularly for new and untried remedies or those of doubtful efficacy, and even those which chemically and pharmacologically appear to be inert or inactive.

6. Relation to true anaphylaxis and anaphylactic shock

It is unnecessary here to discuss the merits and demerits of the numerous theories of anaphylaxis and anaphylactic shock. A summary of most of these may be found in a paper by Dale (28), and some of these have been recently discussed in a monograph by Besredka (29). A close scrutiny of the following reveals that these have no points in common with such colloidal agents as agar, starch, dextrin, acacia, etc., or are sufficiently objectionable on other grounds, abounding in postulated substances and mechanisms; apotoxin theory of Richet (30), anaphylatoxin theory of Friedberger (31), physical theory of Doerr and Moldovan (32), ferment theories of Vaughan (33) and of Jobling and Petersen (34), physical nervous theory of Besredka (29) and peptone theory of Biedl and Kraus (35). The following theories, or explanations, however, require some consideration because they possess certain points of contact with or are based on the type of agents studied by us.

Bronchial theory of Auer and Lewis (36). The marked bronchoconstriction which occurs prevents egress of air during the expiratory phase and the lungs remain distended, the animal dying of asphyxial symptoms and constituting those of anaphylactic shock. Shock and death, though not necessarily all of the symptoms, are prevented by atropine. The bronchial muscle itself (after vagus degeneration) is hyperexcitable. Schultz (37) and Dale (28) showed that the intestinal and uterine muscles of sensitized guinea-pigs share the increased excitability. These phenomena are so constant in truly anaphylactic animals that no investigation is complete without giving them due consideration. In fact, it may be said that the pulmonary phenomena are *the* one set of phenomena, clearly and easily recognizable in guinea-pigs occurring both in true anaphylactic shock and the disturbances from the intravenous injection of several foreign agents, notably agar, congo red, acacia and others studied by us. However, agar alone causes inflation of the perfused lung. This will be referred to again later.

Taraxy theory of Novy and DeKruif (38). This is intended to correlate and explain the effects ("non-specific anaphylactic shock") produced by the intravenous injection of a variety of agents, namely, bacteria, trypanosomes, organ cells, organ extracts, peptone, agar, starch, inulin, kaolin, silicic acid, barium sulphate, etc. A matrix of the poison in the circulating plasma and serum is presupposed and this is called "taraxigen" (from "taraxy," meaning disturbed state or condition from the poison), and its tautomeric poisonous form is "taraxin." The transformation of fibrinogen to fibrin may be likened to the change supposed to take place. A sudden formation of, or too much, "taraxin" brings the explosive effects such as the reactions to foreign agents, and, it is suggested, even the symptoms of eclampsia. Chronic "taraxy" or anaphylactic poisoning is believed to exist in arsphenamine poisoning. The administration of bicarbonate is advocated as a treatment for "taraxies." In specific anaphylactic shock the inducing substance is formed by the interaction of antigen with its antibody. It does not appear to us that the introduction of new terminology and further assump-

tions will materially assist in the explanation of so many diverse conditions.

von Behring's platelet theory (39). The symptoms of anaphylactic shock are ascribed to cerebral capillary embolisms produced by the clumping of platelets. Some disturbance in their medium or themselves causes the platelets to clump and consequently obstruct the smallest blood vessels. Dale and Laidlaw (40) recently observed an abnormal abundance and clumps of agglutinated platelets in the blood of cats injected with histamine. The presence of thrombi and conglutination of corpuscles in the pulmonary vessels was commonly observed by us with the majority of agents injected, but only in one case was there a precipitation of platelets noticed, though, of course, there would be no justification for assuming the liberation of histamine with our agents.

Histamine or histamine-like substances. The recent demonstration of the presence of histamine in peptone (Witte) by Abel amd Kubota (41) would appear to fortify the peptone theory of Biedl and Kraus (35) and a view formerly held by Dale and Laidlaw (42). However, the presence and quick elaboration of a peptone or histamine-like substance still remain to be demonstrated in the anaphylactic shock of guinea-pigs, and its elaboration by non-protein agents like agar, dextrin, etc., would be still more doubtful unless any kind of endothelial injury as suggested by Dale and Laidlaw might do so. In that case the various agents studied by us and anaphylaxis might be shown to enjoy a mutual relationship. However, at present the facts are too limited to justify any such generalization.

Pseudoanaphylaxis. Kritchewsky (6) elaborated a theory on the basis of a parallelism between agglutinating power and ability to cause shock, but this was based only on the power of cotyledon juice to agglutinate bacteria and corpuscles, and precipitate serum. Besides bronchoconstriction and vascular spasm, a type of anaphylactic reaction due to a plugging of pulmonary blood vessels with thrombi and agglutinated corpuscle masses is recognized by Manwaring and Crowe (4). This is called "pseudoanaphylaxis." The reactions with the different agents described

by us come closer to this than to any other designation, if anything of the sort is needed at all.

It is seen that some of these theories run along similar channels, but none of them satisfactorily or adequately explains the symptoms and disturbances produced by the injection of the non-protein colloids and other agents in unsensitized animals as reported in this paper. This is probably equally true of anaphylaxis and anaphylactic shock. To recapitulate:

1. Agar, acacia, dextrin, glycogen, starch, inulin and similar colloids are inert and practically non toxic, as far as their inherent chemical properties are concerned.

2. These colloids are not known to elaborate precipitins or toxins or cause precipitation of the blood. On the contrary they could conceivably adsorb toxins and limit their action. Concerning the rôle of agglutination of corpuscles this will be discussed in a subsequent publication.

3. They are non-protein in character.

4. They possess no known irritant or augmentor effects on smooth muscle (bronchi, intestine and uterus). Proof of this will be submitted later.

5. They are not ferments nor act as such, neither are they known to disturb ferment-antiferment balance. Their action is too rapid.

5a. They do not alter the coagulation of blood.

6. They have no known effects on nerve centers, trunks, endings, ganglia, myoneural junctions, etc., directly.

7. The effects of organic arsenicals like arsphenamine, etc., can be adequately explained by the well known actions of arsenic on the circulation, and imperfections in the technique of administration.

8. The fact that the majority (20 out of 31) of the agents studied by us can be classified as having injured the circulation or the circulation and respiration, the latter being involved as a result of circulatory injury, indicates that no other mechanism such as anaphylaxis or anaphylactic shock need be invoked.

9. Furthermore, no previous treatment or sensitization of the animals with these agents is necessary. Sensitization is a sine

qua non requirement for true anaphylactic shock, requiring at least eight days whatever the dosage of serum according to Besredka. Hence it does not appear that desensitization or anti-anaphylaxis can be invoked or regarded as part of the mechanism, a condition Besredka is justified in raising against claims of relationship between anaphylaxis and disturbances produced by various foreign agents (starch, kaolin, agar, etc.).

10. It has even been doubted if these theories explain the responses to injections of peptone and heterologous serums, whose actions present many similarities to, but lack some of the characteristics of anaphylactic poisoning (Loewit (44); Karsner (45)).

Conclusion. When taken together with the explanations supplemented throughout the text, our results indicate that it is quite erroneous to regard the disturbances produced by the intravenous injection of sundry agents in the same category with true anaphylaxis, or bearing any causal relationship to it whatsoever or vice versa.

7. *Specific gravity of collapsed and distended lungs*

This is intended as a supplement to the work reported in this paper. Sometimes it is difficult to correctly interpret distention or inflation of the lungs in anaphylactic shock. It is well known that collapsed and even edematous and hemorrhagic and, of course, distended lungs of guinea pigs will generally float on water. Therefore, some lighter medium was sought by which it might be possible to differentiate collapsed and distended lungs. Ordinary ethyl ether of specific gravity ranging from 0.711 to 0.716 out of a number of fluids of low specific gravity was found to answer the purpose very well. This is indicated by the results with 114 different lungs of guinea-pigs used in our experiments and recorded in table 3.

Observations on 107 different lungs indicated that when their specific gravity was increased with the degree of congestion encountered these were all lighter than water, but heavier than ether when collapsed and congested (31 out of 32 lungs). When even a moderate degree of distention accompanied by moderate

congestion was present, about 77 per cent of 74 lungs tested floated on ether, and all or 100 per cent of the 5 tested floated when distention alone was present. Two collapsed lungs without congestion and hemorrhages sank in ether and floated on water. That is, a lung partially or markedly distended, in other words, partially or wholly anaphylactic, is lighter (floats) than ether, while a lung that is simply congested, or collapsed and congested (not anaphylactic), is heavier than ether. We found this test of some value in interpreting the results of our work.

TABLE 3

Specific gravity of collapsed lungs

CONDITION OF LUNGS	NUMBER OF LUNGS TESTED	LIGHTER THAN ETHER (FLOAT)		HEAVIER THAN ETHER (SINK)		LIGHTER THAN WATER	
		Number	Per cent	Number	Per cent	Number	Per cent
Distension alone	5	5	100			5	100
Collapse alone	2			2	100	2	100
Collapse and congestion	32	1	3	31	97	29*	100
Distension and congestion	74*	57	77	17	23	75	100
Total number of lungs tested	114†						

* No more lungs were tested.

† One more added from column "Lighter than water."

Conclusion. Ether immersion appears to be a simple test for distinguishing collapsed and congested lungs from distended and congested lungs possessing a moderate to a strong degree of distention.

IV. CONCLUSIONS

1. Mild ether anesthesia and the intravenous injection of normal saline are practically harmless to the circulatory and respiratory systems of guinea-pigs, but the remaining 29 of the 31 agents studied were found to be distinctly harmful in varying degrees.

2. The injury produced by the intravenous injection of the following agents is expressed through the respiratory system chiefly, producing definite anaphylactoid symptoms, and pulmonary distention, congestion, hemorrhages and thrombi with-

out definite cardiac dilatation; agar sol (0.5 per cent), agar gel (0.5 per cent), agar sol-gel (1 : 4 and 1 : 6), dog's bile, human serum and typhobacterin. Pending further proof, the mechanism of agar action may be stated to consist of passive bronchoconstriction (really compression of bronchi) with consequent phenomena of asphyxia. Consequently, the disturbances of intravenous administration following the injection of various agents in unsensitized animals do not necessarily constitute true anaphylaxis or anaphylactic shock.

3. The following agents when injected intravenously were found to produce definite symptoms of respiratory distress together with pulmonary congestion and hemorrhages, but, as a rule, no distention and thrombi; althea extract, arsphenamine, collargol, congo red, dextrin, gelatin, inulin, neoarsphenamine, phylacogen (mixed infection), rabbit's serum, sodium arsenate and venarsen. The injuries produced appear to be principally concerned with the circulatory system. The intravenous administration for therapeutic purposes of such agents as collargol and phylacogen, which still lack a scientific basis, is unjustified.

4. The intravenous injection of the following agents injures the circulatory and respiratory systems as indicated by the presence of anaphylactoid symptoms, pulmonary distention, congestion and hemorrhage together with cardiac dilatation; acacia, plain, dialyzed and toxified with rabbit's serum, beef serum, glycogen, nuclein solution, pancreatin, peptone (large and very small doses), pollen extracts and starch. Thrombi in pulmonary vessels occurred after the injection of acacia in therapeutic doses and concentrations, and pollen extracts. The indiscriminate and careless administration of acacia and peptone intravenously should be enjoined with caution.

5. The results obtained are decidedly against the promiscuous and unwarranted use of the intravenous method of administering drugs as a routine therapeutic measure, particularly for new and untried remedies or those of doubtful efficacy, and even those which chemically and pharmacologically appear to be inert or inactive.

6. When taken together with the explanations supplemented throughout the text, our results indicate that it is quite erroneous to regard the disturbances produced by the intravenous injection of sundry agents in the same category with true anaphylaxis or bearing any causal relationship to it whatsoever, or vice versa.

7. Ether immersion appears to be a simple test for distinguishing collapsed and congested lungs from distended and congested lungs possessing a moderate to a strong degree of distention.

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REFERENCES

(1) Friedberger and Tsuneoka: Ztschr. f. Immunitäts f. u. exp. Therap., xx, 405.
(2) Nathan: Ztschr. f. Immunitätsf., 1914, xxiii, 204.
(3) Bordet: Compt. rend. soc. de. biol., 1913, lxxiv, 225.
(4) Novy and DeKruif: J. Inf. Dis., 1917, xx, 536.
(5) Novy, F. G., DeKruif and Novy, F. O.: J. Inf. Dis., 1917, xx, 657.
(6) Kritchewsky: J. Inf. Dis., 1918, xxii, 101. Russkiy Vratch, 1916, xv, No. 4. Bronfenbrenner: Proc. Soc. Exp. Biol. Med., 1915, xiii, 19.
(7) Danysz: Ann. de l'Inst. Pasteur, 1917, xxi, 114.
(8) Hanzlik, Karsner and Fetterman: J. Pharm. Exp. Therap, 1919, 14: 229.
(9) Karsner: Centralbl. f. Bakt. Parasiten. u. Infektionskrankeiten, 1911, lxi, 247.
(10) Ono: Acta Scholae Med. Univ. Imp. Kioto, 1918, ii, 271.
(11) Stokes: J. Am. Med. Assoc., 1919, lxxii, 241.
(12) Milian: Bull. soc. francaise de dermat. et de syph. 1912, xxiii, 520; 1913, xxiv, 272 and 436.
(13) Berman: Arch. Int. Med., 1918, xxii, 217.
(14) Beeson: Am. J. Syphilis, 1919, iii, 129.
(15) Hirano: Kitasato Arch. Expt. Med., 1919, iii, 1.
(16) Jackson and Smith: J. Pharm. Exp. Therap., 1918, xii, 221.
(17) Auer: J. Exp. Med., 1911, xiv, 497.
(18) Hirano: Japanese Med. World, 1919, June 22.
(19) DeKruif and German: J. Inf. Dis., 1917, xx, 833.
(20) Sorev: Compt. rend. soc. biol., 1918, lxxxi, 57; Ibid, 1918, lxxxi, 563.
(21) Launoy: Compt. rend. soc. biol., 1918, lxxxi, 164.
(22) DeKruif: Annals of Surgery, 1919, lxix, 297.
(23) Kruse: Am. J. Physiol., 1919, xlix, 137 (Proc).
(24) Wago: Arch. Int. Med., 1919, xxiii, 251.
(25) Nolf: J. Am. Med. Assoc., 1919, lxxii, 1901.
(26) Okasaki: Chem. Absts., 1919, xiii, 45.
(27) Stewart, G. N.: Amer. J. Physiol., 1919, xlix, 233.

(28) Dale: J. Pharm. Exp. Therap., 1913, iv, 167.
(29) Besredka: Anaphylaxie et Antianaphylaxie, 1917, Masson et Cie., Paris.
(30) Richet: L'Anaphylaxie, Paris, 1912.
(31) Friedberger: Zeitschr. f. Immunitätsf., 1910, iv, 636.
(32) Doerr and Moldovan: Biochem. Zeitschr., 1912, xli, 27.
(33) Vaughan: J. Inf. Dis., 1907, iv, 476.
(34) Jobling and Petersen: J. Exp. Med., 1914, xxii, 468.
(35) Biedl and Kraus: Wein. Klin. Wochschr., 1910, xxii, 363.
(36) Auer and Lewis: J. Am. Med. Assoc., 1909, liii, 458; J. Exp. Med., 1910, xii, 151.
(37) Schultz: J. Pharm. Exp. Therap., 1910, i, 549; Hyg. Lab. Bull., U. S. P. H. S. 1912, No. 80.
(38) Novy and DeKruif: J. Am. Med. Assoc., 1917, lxviii, 1524.
(39) von Behring: Deutsch. med. Wochsch., 1914, xl, No. 41.
(40) Dale and Laidlaw: J. Physiol., 1919, lii, 355.
(41) Abel and Kubota: J. Pharm. Exp. Therap., 1919, xiii, 243.
(42) Dale and Laidlaw: J. Physiol., 1910, xli, 318.
(43) Manwaring and Crowe: Proc. Soc. Exp. Biol. Med., 1917, xiv, 100.
(44) Loewit: Arch. f. Exp. Path. Pharm., 1913, lxxiii, 1.
(45) Karsner: Zeitschr. f. Immunitätsf., u. exp. Therap., 1912, xiv, 81.

A COMPARISON OF THE PROPHYLACTIC EFFECTS OF ATROPINE AND EPINEPHRINE IN ANAPHYLACTIC SHOCK AND ANAPHYLACTOID PHENOMENA FROM VARIOUS COLLOIDS AND ARSPHENAMINE[1]

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I. INTRODUCTION

The results previously reported by us (1) with the intravenous injection of agar and other non-protein colloids and also the organic arsenicals, including arsphenamine, into guinea-pigs indicated that these bear no relationship to anaphylaxis or anaphylactic shock. Concerning agar and some other agents further proof is necessary to establish whether or not an actual stimulation of the bronchi exists in the accompanying pulmonary distension. This could be obtained in part, at least, by the use of atropine and epinephrine as bronchodilators in the prophylactic treatment of the distension.

That is, if the distension is due to active bronchoconstriction, such as in anaphylactic shock, this should be prevented or relieved by atropine as originally shown by Auer (2). Atropine also antagonizes the bronchoconstrictor action of peptone in intact guinea-pigs (Biedl and Kraus (3)), and of both peptone and histamine in perfused lungs (Baehr and Pick (4)). The dose

[1] This investigation was supported in part by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

of atropine bears a quantitative relationship to the size of the intoxicating dose of protein (5).

With epinephrine the effects are more complicated and not so certain, depending in part on the functional state of the bronchiolar musculature (Januschke and Pollak (6)) and in part on the time of administration, since the action is fleeting. Januschke and Pollak found that epinephrine relaxes the untreated bronchi somewhat and the greatest bronchodilator action was obtained when the bronchi were previously constricted by peptone and muscarine, but not by histamine and ergotoxin. Epinephrine also relieves the bronchoconstriction by peptone in guinea-pigs (Biedl and Kraus (3)) and that produced by peptone and histamine in perfused lungs when high concentrations are used (Baehr and Pick (4)).

It is apparently on the basis of their bronchodilator effects (perhaps circulatory, in part) that the use of epinephrine is recommended by Milian (7), Beeson (8) and Hirano (9) and atropine by Stokes (10) in the treatment of the "nitritoid crises" of arsphenamine poisoning, which is regarded by Stokes as a form of anaphylaxis. Nolf (11) advises the use of epinephrine in connection with the disturbances accompanying proteose therapy. Whatever the clinical success with the use of these drugs, experimentally, at least, the basis has not been clearly established. It does not necessarily follow that whatever beneficial effects they may have are due to their action on the bronchioles.

We have, therefore, attempted to test out this and also other features of anaphylactoid reactions. The main objects of the work may be stated as follows: (1) Prophylactic treatment of disturbances accompanying the administration of arsphenamine, peptone and non-protein colloids experimentally in guinea-pigs by means of atropine and epinephrine; (2) elucidation of the mechanism of pulmonary distension with agar by means of these agents; (3) treatment of anaphylactic shock in serum-sensitized guinea-pigs with epinephrine, for, so far as we know, this has not been tried. In dogs Pelz and Jackson (12) found epinephrine to relax the constricted bronchi in anaphylactic shock if injected early.

II. METHODS

Because of the pulmonary effects, which we were chiefly investigating, guinea-pigs were chosen as the most suitable animals. These were injected intravenously in the same way as described in the previous paper. The various agents used to produce the respiratory disturbances were prepared in exactly the same way also. These served as controls for the experiments with atropine and epinephrine reported in this paper. For description of the results with these, as well as the methods used, the previous paper (1) should be consulted. It is only necessary to describe here the method of administering atropine and epinephrine.

Atropine sulphate (0.1 per cent) was always injected intravenously and allowed to act about five minutes before the administration of the agent producing anaphylactoid symptoms and in the dosage of about 1 mgm. per 100 grams of animal. The usual effects of increased respiratory rate and hyperexcitability were observed.

Epinephrine was used intravenously in two ways: (1) Immediately preceding, and (2) together with the agent to be observed. The dosage was that which produces a definite rise of blood pressure in dogs, namely, 0.5 cc. of 1 : 10,000 per kilo or 0.0005 cc. of 1 : 10,000 per gram of guinea-pig. Prompt effects were produced as indicated by the marked increase in respiratory and pulse rate. The following agents were studied: arsphenamine, agar, dextrin, congo red, peptone, beef serum, typhobacterin, acacia, pollen extract, starch, althea and serum-sensitized animals.

III. DISCUSSION OF RESULTS

The data pertaining to individual experiments with the different agents are presented in tables 1 and 2. The descriptions of the controls, that is, effects of different agents without atropine and epinephrine as well as normal saline, have been presented in a previous paper (1) and are omitted in this paper to save space. Instead, a comparison of all the changes obtained with the different agents in the present series has been made with those of the controls and the whole is conveniently summarized in table 3.

TABLE 1

Treatment of anaphylactoid phenomena from various agents with atropine

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM GUINEA PIG	PRINCIPAL SYMPTOMS	FATE OF ANIMAL	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Dextrin (6 per cent)							
	grams	*cc.*	*mgm.*		*minutes*		
50	250	Atropine 3 mgm. 6 per cent dextrin 3 cc.	0.012 0.61	Slight dyspnea, otherwise normal	Killed, 30½	Marked pulmonary distention and minute hemorrhages, slight cardiac and abdominal congestion	Marked distention, marked congestion, marked hemorrhage
111	500	Atropine 8 mgm. Dextrin 3 cc. of 10 per cent	0.016 0.6	Marked increase in respiration; restlessness	Killed, 52	Marked pulmonary distention and congestion and slight hemorrhages; very marked cardiac dilatation; moderate abdominal congestion; blood clotted in 4 minutes	Moderate distention, marked congestion, slight hemorrhage
Agar sol (0.5 per cent)							
55	330	Atropine 3 mgm. 3.0 cc. of 0.5 per cent agar	0.0099 0.046	Dyspnea; marked restlessness; cyanosis marked; depression marked, respiration slow	Killed, 31	Lungs distended and congested; marked cardiac dilatation, abdominal congestion	Moderate distention, moderate congestion, slight hemorrhage, marked thrombosis

Agar sol gel (1:6)

60	230	Atropine 3 mgm. 5 cc. agar	0.013 0.018	Dyspnea; respiration slow	Killed, 30	Lungs, distended, hemorrhages, no edema; slight cardiac dilatation, slight abdominal congestion	Marked distention, marked congestion, marked hemorrhage
97	430	Atropine 6 mgm. 5 cc. of agar	0.014 0.01	Marked dyspnea respiration increased, depression marked; recovery from symptoms	Killed, 42	Marked pulmonary distention, congestion and hemorrhages; marked cardiac dilatation, abdominal congestion and gastric submucous hemorrhages; rapid blood coagulation	Moderate distention, marked congestion, slight hemorrhage, marked thrombosis
98	365	Atropine 6 mgm. Agar 8 cc.	0.017 0.019	Markedly increased respiration; no dyspnea	Killed, 33	Very marked pulmonary distention, hemorrhage and congestion; moderate cardiac dilatation; slight abdominal congestion; blood clots rapidly	Moderate distention, marked congestion, marked hemorrhage
92	250	Atropin 4 mgm. Agar 5 cc.	0.016 0.017	Marked dyspnea; increased respiration; restlessness at first, later amelioration of all symptoms	Killed, 33	Moderate pulmonary distention, hemorrhage and congestion; cardiac dilatation; abdominal congestion; blood clots rapidly	Moderate distention, marked congestion

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM GUINEA PIG	PRINCIPAL SYMPTOMS	FATE OF ANIMAL	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Congo red (1 per cent)							
	grams	*cc.*	*mgm.*		*minutes*		
118	330	Atropine 4 mgm. Congo red 3 cc.	0.0091 0.091	Increased respiration; excitable; quiet	Killed, 43	Lungs collapsed and congested; no hemorrhages; abdominal congestion; slight cardiac dilatation; blood clotted in 4 minutes	Marked congestion, slight hemorrhage
156	470	Atropine 5 mgm. Congo red 3 cc.	0.0107 0.062	Increased respiration; later marked depression	Fatal, 20	Lungs collapsed and markedly congested; marked cardiac dilatation and abdominal congestion	Marked congestion, slight local distention
Horse serum sensitization							
48	200	Atropine 3 mgm. Serum 0.5 cc., diluted to 3 cc.	0.015 0.0025 cc.	Marked increase in respiratory rate; dyspnea (?); increased motor excitability	Killed, 37	Slightly distended lungs, pneumonia right base; slight cardiac and abdominal congestion; 20th day sensitization	Moderate distention, moderate congestion

125	490	Atropine 5 mgm. Serum 0.8 cc.	0.0102 0.0017	Increased respiration; restlessness; no dyspnea	Killed, 42	Considerable pulmonary distention, marked congestion, no hemorrhages; moderate cardiac dilatation, no abdominal congestion; blood clotted in 4 minutes	Moderate distention, moderate congestion
				Beef serum			
117	450	Atropine 5 mgm. Beef serum 2 cc.	0.011 0.0044 cc.	Increased respiration	Killed, 30	Moderate pulmonary distention; slight hemorrhage and moderate congestion; moderate cardiac dilatation and abdominal congestion; blood clotted in 3 minutes	Marked distention, marked congestion; marked hemorrhage
159	470	Atropine 5 mgm. Beef serum 2 cc.	0.0106 0.0042 cc.	Restlessness	Killed, 43	Lungs practically collapsed, congested and hemorrhages; no cardiac dilatation and abdominal congestion	Slight congestion; slight local distention, practically normal

TABLE 1—*Continued*

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM GUINEA PIG	PRINCIPAL SYMPTOMS	FATE OF ANIMAL	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
				Peptone (10 per cent)			
	grams	*cc.*	*mgm.*		*minutes*		
112	540	Atropine 6 mgm. Peptone 0.2 cc.	0.011 0.037	Increased respiration; restlessness	Killed, 35	Pulmonary distention; small hemorrhages and moderate congestion; slight cardiac dilatation and moderate abdominal congestion; blood clots in 2 minutes	Moderate distention, marked congestion
158	280	Atropine 3 mgm. Peptone 0.016 cc.	0.011 0.0058	Restlessness; respiration increased	Killed, 57	Lungs collapsed and congested with few hemorrhages; moderate cardiac dilatation	Slight congestion, slight local distention, practically normal
				Soluble starch (6 per cent)			
119	520	Atropine 5 mgm. Starch 3 cc.	0.0096 0.34	Increased respiration, and dyspnea moderate	Killed, 44	Marked pulmonary distention, slight congestion and hemorrhages; slight cardiac dilatation and abdominal congestion; blood clotted in 3 minutes	Marked congestion

			Althea (15 per cent extract)				
124	545	Atropine 4 mgm. Althea 3 cc.	0.0091 0.083	Increased respiration; moderate dyspnea	Killed, 47	Slight pulmonary distention, marked congestion, no hemorrhages; slight cardiac dilatation; slight abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion
160	400	Atropine 5 mgm. Althea 3 cc.	0.0125 1.25	Increased respiration	Killed, 38	Lungs collapsed, congested; punctate hemorrhages; no cardiac dilatation and no abdominal congestion	Moderate congestion marked hemorrhage, marked edema, marked general distention
			Arsphenamine (0.5 per cent)				
106	270	Atropine 6 mgm. Arsphenamine 0.4 cc.	0.022 0.008	Increased respiration; some restlessness	Killed, 40	Lungs collapsed, slight congestion, not hemorrhagic; slight cardiac dilatation; moderate abdominal congestion	Moderate distention, marked congestion, hemorrhage, thrombosis
122	410	Atropine 4 mgm. Arsphenamine 0.7 cc.	0.01 0.0085	Increased respiration	Killed, 31	Lungs distended, congested and hemorrhagic; moderate cardiac dilatation; slight abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion, hemorrhage, conglutination thrombi

TABLE 1—*Concluded*

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM GUINEA PIG	PRINCIPAL SYMPTOMS	FATE OF ANIMAL	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
				Typhobacterin (Mulford)			
	grams	*cc.*	*mgm.*		*minutes*		
114	400	Atropine 5 mgm. Typhobacterine 2 cc.	0.0125 0.005	Slight dyspnea, restlessness	Killed, 32	Moderate pulmonary distention and congestion, no hemorrhages; moderate cardiac dilatation; no abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion, slight hemorrhage, thrombosis
				Acacia (6 per cent)			
116	530	Atropine 6 mgm. Acacia 5 cc.	0.0113 0.57	Some dyspnea; respiration increased	Killed, 30	Marked pulmonary distention, moderate congestion and marked hemorrhages; moderate cardiac dilatation and slight abdominal congestion	Slight distention, marked congestion, hemorrhage
120	680	Atropine 6 mgm. Acacia 8 cc.	0.0088 0.7	Increased respiration; moderate dyspnea, restlessness	Killed, 39	Slight pulmonary distention, marked congestion and few small hemorrhages; moderate cardiac dilatation and abdominal congestion; blood clots in 2 minutes	Slight distention, marked congestion

Pollen extract (hay fever fall)

113	440	Atropine 8 mgm. Pollen extract 1 cc. = 0.02 mgm. nitrogen	0.018 0.0024 cc.	Restlessness	Killed, 33	Very slight pulmonary distention, marked congestion and very minute hemorrhages; moderate cardiac distension and abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion, marked hemorrhage
121	470	Atropine 3 mgm. (Hay fever pollen extract 1 cc. spring) = 0.02 mgm. nitrogen	0.007 0.0021 cc.	Restlessness, jerky	Killed, 34	Pulmonary distention, moderate congestion, no hemorrhages; moderate cardiac dilatation; no abdominal congestion. Blood clotted in 2½ minutes	Moderate distention, marked congestion, slight hemorrhage, conglutination thrombi

TABLE 2

Treatment of anaphylactoid phenomena from various agents with epinephrine

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM GUINEA PIG	PRINCIPAL SYMPTOMS	FATE OF ANIMAL	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
				Horse serum sensitization			
	grams	*cc.*	*mgm.*		*minutes*		
51	275	Epinephrine = 0.13 After serum = 0.5 cc. to 3 cc. N.S.	0.000048 0.00189 cc.	Marked dyspnea convulsions; typical shock	Fatal, 3	Marked pulmonary inflation and congestion; pulmonary edema; heart slightly dilated; slight abdominal congestion. Sensitized 20th day	Moderate distention, marked congestion, marked hemorrhage
57	220	Epinephrine = 0.1 together with serum = 0.5 cc. to 3 cc. N.S.	0.000046 0.0023 cc.	Marked dyspnea; cyanotic; twitching of ears; ruffling of hair; depression	Killed, 32	Lungs distended; minute hemorrhages; congestion slight, slight cardiac dilatation; marked abdominal congestion	Marked distention
100	270	Epinephrine = 0.013 (0.3 cc. 1:10,000) 3 minutes before Serum 0.5 cc.	0.00005 0.0018 cc.	Convulsions; marked dyspnea; depression; death	Fatal, 5	Marked pulmonary inflation, hemorrhages and congestion; marked cardiac dilatation; no abdominal congestion; blood clotted in 5 minutes. Twenty-second day of sensitization	Marked congestion, slight hemorrhage, conglutination thrombi

101	170	Epinephrine = 0.01 (0.1 cc. 1:10,000) just before Serum 0.5 cc.	0.00006 0.003	Marked dyspnea; restlessness; cyanosis; depression; gradual amelioration of symptoms	Killed, 30	Lungs moderately inflated, markedly congested and hemorrhagic; moderate abdominal congestion; blood clotted in 3 minutes. Animal markedly emaciated. Twenty-second day of sensitization	Marked congestion, marked hemorrhage
Dextrin (6 per cent)							
49	250	Epinephrine = 0.1 of 1:10,000 Dextrin 3 cc.	0.00004 0.61	Marked dyspnea; depression; hair ruffled	Killed, 30	Lungs collapsed and markedly congested with many hemorrhages; cardiac dilatation and slight abdominal congestion	Marked congestion, hemorrhage, edema
Agar sol (0.5 per cent)							
56	205	Epinephrine = 0.1 of 1:10,000 3 cc. 0.5 per cent agar	0.00005 0.073	Marked depression; dyspnea; cyanotic	Fatal, 7	Pulmonary distention and congestion; marked cardiac dilatation; moderate abdominal congestion	Moderate distention, marked congestion, hemorrhage, marked thrombosis, edema
Agar sol gel (1:6)							
61	210	Epinephrine = 0.1 of 1:10,000 5 cc. agar	0.000048 0.02	Marked dyspnea; increased respiration; irritable; air hunger	Killed, 30	Pulmonary distention; edema and congestion, also hemorrhages, distinct cardiac dilatation	Marked distention, marked congestion, marked hemorrhage

TABLE 2—*Continued*

NUMBER	WEIGHT OF GUINEA PIG	TOTAL DOSE INJECTED	DOSE PER GRAM GUINEA PIG	PRINCIPAL SYMPTOMS	FATE OF ANIMAL	AUTOPSY	MICROSCOPIC EXAMINATION OF LUNGS
Agar sol gel (1:6)—*Continued*							
	grams	*cc.*	*mgm.*		*minutes*		
91	250	Epinephrine = 0.13 of 1:10,000 5 cc. agar	0.000052 0.017	Marked dyspnea; convulsions	Fatal, 8	Marked pulmonary distention, congestion and hemorrhages; slight cardiac dilatation, pericardial hemorrhages	Marked distention, marked congestion, marked hemorrhage
Arsphenamine (0.5 per cent)							
162	200	Epinephrine = 0.13 Arsphenamine = 0.6	0.00005 0.012	Respiration increased	Killed, 34	Lungs distended considerably, very hemorrhagic and moderately congested; no cardiac dilatation or abdominal congestion	Marked congestion, slight local distention
107	390	Epinephrine = 0.2 Arsphenamine = 0.68	0.000051 0.0087	Increased respiration; restlessness	Killed, 31	Lungs collapsed, markedly congested and hemorrhagic; moderate cardiac dilatation; very slight abdominal congestion	Slight distention, marked congestion, marked hemorrhage, thrombosis

				Peptone (10 per cent)			
99	430	Epinephrine 0.1 cc. peptone	0.00007 0.023	Markedly increased respiration only	Killed, 30	Pulmonary distention, numerous hemorrhages and congestion; cardiac dilatation and moderate abdominal congestion	Moderate distention, marked congestion, slight hemorrhage, conglutination thrombi
157	550	Epinephrine Peptone = 0.033	0.00005 0.006	Markedly increased respiration	Killed, 50	Lungs inflated markedly congested and hemorrhagic; marked cardiac dilatation and abdominal congestion	Marked congestion, marked hemorrhage, marked edema, conglutination thrombi, distention of unfilled alveoli
				Soluble starch (6 per cent)			
161	300	Epinephrine = 0.15 Starch = 0.18	0.00005 0.6	Marked increase in respiration; hair ruffled	Killed, 36	Lungs moderately distended, markedly congested and hemorrhagic; cardiac dilatation; moderate abdominal congestion	Moderate congestion, slight hemorrhage, slight local distention, practically normal

* Takamine preparation; slightly precipitated.

TABLE 3

Summary of preventive power of atropine and epinephrine against anaphylactoid phenomena from various agents injected intravenously as compared with controls without treatment

COMPLETE*	PARTIAL	NONE
Prevention by atropine		
Beef serum, whole Peptone, 10 per cent; small dose	Agar sol, 0.5 per cent (not fatal, otherwise no change)† Agar sol gel, 1:6 (fewer thrombi, otherwise same, doses larger; partial prevention to a certain extent) Arsphenamine, 0.5 per cent (symptoms less severe, otherwise same; thrombi present) Beef serum, whole (not fatal) Congo red, 1 per cent (symptoms less severe; no distention) Peptone, 10 per cent; larger dose (not fatal, otherwise same) Serum-sensitization (not fatal; symptoms and autopsy changes less severe) Typhobacterin, Mulford (symptoms less severe, otherwise same)	Acacia, 6 per cent (same) Althea extract, 15 per cent (about same) Dextrin, 6 per cent (worse, more distention) Pollen extract, hay fever fall, Mulford (about same, perhaps dyspnea less) Starch (about same)
Prevention by epinephrine		
	Arsphenamine, 0.5 per cent (less dyspnea, otherwise same) Serum-sensitization [not fatal, more pulmonary hemorrhage, otherwise same, epinephrin injected with antigen in one, and just preceding (less than 2 minutes) in the other animal]	Agar sol, 0.5 per cent (same; fatal) Agar sol gel, 1:6 (same; fatal) Dextrin, 6 per cent (same) Peptone, 10 per cent, small doses (worse; thrombi and hemorrhages; otherwise same) Serum-sensitization (fatal; epinephrine injected after antigen in one, and 3 minutes before in another animal) Starch, 6 per cent (same)

* Means complete recovery as to absence of symptoms and autopsy, and microscopic changes in lungs, and approaching normal.

† Remarks in parenthesis refer to differences from controls injected with the same kind of agent and dose without atropine or epinephrine as the case may be.

1. Prevention by atropine

From the summary in table 3, it is seen that atropine in the dosage of 0.01 mgm. per gram of animal, equivalent to 600 to 1000 times the average therapeutic dose for man, acts favorably as a preventive for the effects of beef serum and peptone. Complete prevention as to symptoms, autopsy and microscopic changes were obtained with beef serum in one animal and somewhat less in another. The usual effects of a small dose of peptone, equivalent to the therapeutic dose recommended by Nolf (11) in the treatment of infectious and febrile conditions, were completely prevented by atropine. The protection was not so complete with a dose once as large; but death, at least, was prevented.

The partial protection afforded by atropine against the effects of agar sol, agar sol-gel, arsphenamine, congo red, serum-sensitization and typhobacterin was concerned principally with diminution in the severity of symptoms. Fatalities from agar sol, agar sol-gel and the anaphylactic (serum-sensitized) animals were prevented. So far as autopsy and microscopic changes are concerned there was practically no difference from the controls. The partial prevention from the effects of beef serum and peptone have been discussed above.

Concerning agar sol-gel, the interpretation of "partial prevention" by atropine is perhaps liberal. That is, the controls received a small dosage, while in the present experiments the dosage was practically fatal as shown by the results of Novy and DeKruif (12) and our own experiments with agar sol-gel and epinephrine, in which fatalities occurred with the same dosage. For this reason, the results with atropine are interpreted as indicating some protection (as to fatality), and there is certainly no doubt about this with agar sol. However, the atropine did not prevent the general effects of agar sol-gel, namely, symptoms of respiratory distress, marked pulmonary distention, hemorrhages and thrombi, although the thrombi were somewhat less conspicuous. These experiments, therefore, so far as the mechanism of agar sol-gel action is concerned, are indecisive as originally hoped

for. The comparisons which we hoped to obtain with various smooth muscle (bronchiolar) stimulants, namely, peptone, beef-serum and serum-sensitized animals, are too variable to permit the free use of these, although the tendency is favorable with beef serum and peptone. That is, complete prevention was obtained with these augmentors of smooth muscle (bronchiolar, etc.) in some animals and this was certainly more than was obtained with agar, indicating that the mechanism of agar action is different from peptone and similar augmentors of plain muscle. This would mean that the anaphylactoid effects of agar are not like or partake of true anaphylactic shock. However, this statement is made reservedly considering the variability of results obtained with the smooth muscle stimulants in this paper.

This is even more true of the results with epinephrine and agar sol-gel presently to be discussed. Because of this, and a certain amount of variability in the response of guinea-pigs to the various agents that have been tried, including agar, it is manifestly impossible to settle the question of how agar acts merely by experiments with intact animals. The matter can finally be settled by perfusion of the lungs. In anticipation of results along this line to be published later, it can be stated that agar acts differently from the various smooth muscle stimulants and, therefore, bears no relation to anaphylactic shock.

A word is necessary as to the mechanism of the partial amelioration obtained with atropine in the majority of our experiments. The following possibilities exist; (1) sufficient (moderate or even less) bronchial relaxation due to the atropine with prevention of the fatal effects of asphyxia; (2) improvement in or diminished circulatory disturbances such as cardiac dilatation, from paralysis of the parasympathetic endings by atropine; (3) respiratory stimulation by atropine with consequent amelioration of asphyxia; (4) ineffective central parasympathetic stimulation from asphyxia owing to paralysis of vagus endings by atropine. Whether any particular one or all of these factors are operative we do not know, and the conditions were not suitable for investigation of this.

However, it is quite patent that mere bronchial relaxation from atropine need not be assumed as responsible for the beneficial effects of relief that it may give, particularly with such a drug as arsphenamine, and probably also of congo red. If this is true, the further assumption that the arsphenamine reactions are a form of anaphylactic shock is certainly unjustified. This is believed to be the case. The results reported in our previous paper fortify this conclusion, since arsphenamine did not at all produce effects resembling those of anaphylaxis or anaphylactic shock. On the contrary, the disturbances were regarded to be of circulatory origin and, therefore, any amelioration that atropine may give is to be attributed to an improvement in the circulation rather than the supposed bronchiolar effects, at least in guinea-pigs. There is no good reason to believe that human individuals behave differently.

Atropine was found to give no demonstrable relief from the symptoms and disturbances produced by acacia, althea, dextrin, pollen extract and starch. Because of this and the reasons presented in our previous paper it is reasonable to conclude that the effects of these agents bear no relationship to anaphylaxis or anaphylactic shock.

Conclusions. The intravenous injection of atropine in guinea-pigs in the dosage of 0.01 mgm. per gram of body weight can completely prevent the toxic effects produced by the intravenous injection of beef serum, and peptone in doses corresponding to those used in the proteose therapy of Nolf. Partial protection was obtained against the effects (principally symptoms of respiratory distress) of agar sol, agar sol-gel, arsphenamine, congo red, peptone (large doses), serum-sensitization (anaphylactic shock) and typhobacterin. No protection was obtained against the effects of acacia, althea, dextrin, pollen extract and starch.

2. *Prevention by epinephrine*

As indicated by the summary in table 3, protection with epinephrine against the anaphylactoid symptoms produced by various agents is even less than with atropine. Epinephrine itself

(in the dosage used) produces rather profound effects such as increased respiration and pulmonary hemorrhages. Therefore, the experiments were limited to a number of the more important agents, namely, agar sol, agar sol-gel, dextrin, peptone, serum sensitization, starch and arsphenamine.

Partial protection was obtained only against 2 of these, namely arsphenamine and in serum-sensitization (anaphylactic shock). In the arsphenamine animals there was merely some amelioration of symptoms, the remaining effects being present as in the controls. This amelioration is probably mainly concerned with the circulation resulting in relief from the asphyxia due in turn to the depressant action of arsenic as in untreated animals. The effects of epinephrine on previously untreated (unconstricted) bronchioles being indeed doubtful, and this taken together with the possibilities indicated above with atropin, and the fact that the effects of arsphenamine in control animals do not resemble those of true anaphylactic shock, confirms the conviction that whatever beneficial effects epinephrine may have in the disturbances from arsphenamine are concerned with improvement in the circulation. The burden of proof rests on those who attribute the benefits to bronchiolar relaxation, and consequently amelioration of anaphylactic shock or anaphylaxis.

It is seen that the protective powers of epinephrine in true anaphylactic shock vary with the time of administration of the drug (see tables 2 and 3). In two animals death was prevented when the epinephrine was injected together with and just preceding the injection of the antigen (horse-serum). On the other hand, when a greater interval elapsed between the injection of epinephrine and antigen, the two animals injected died of anaphylactic shock in the usual way. In one animal the epinephrine was injected three minutes before and in the other some time after the antigen when the conditions for eliciting the symptoms of anaphylactic shock were under mobilization and leading to a fatal issue from the bronchiolar spasm. These results agree perfectly with the well known pharmacological actions of epinephrine on the bronchi. That is, epinephrine is effective as a dilator only on constricted bronchioles, when it is present in effective

concentration, which must be at the time constriction is occurring or just preceding it. After the bronchiolar constriction has occurred, as it does rather violently and with remarkable rapidity in anaphylactic shock, epinephrine is no longer active because a fatal issue has practically supervened. Larger doses might perhaps still be effective, but these were practically precluded in our experiments with guinea-pigs because of the extensive pulmonary hemorrhages produced by epinephrine itself and the consequent complications. As indicated by the results obtained, epinephrine is effective as a prophylactic in anaphylactic shock of guinea-pigs when injected together with the antigen or immediately preceding it.

These results with epinephrine in anaphylactic shock throw some light on the mechanism of agar action and similar colloids. We have seen how epinephrine acts as a prophylactic in anaphylactic shock when injected under proper conditions, and this is due, no doubt, to an alleviation of the bronchoconstriction as indicated by the diminished pulmonary distention at autopsy, and other effects. Epinephrine was injected in the same way in the experiments with agar, but practically no protection whatsoever was obtained. In fact, certain effects (pulmonary hemorrhages) were accentuated. The fatalities were greater. This is so because the mechanism through which epinephrine exerts its beneficial effects is absent in agar action, namely, active bronchoconstriction. Therefore, the pulmonary distention from agar and similarly acting agents cannot be due to the same cause as in anaphylaxis or anaphylactic shock.

Besides being inefficient against agar, epinephrine furnished no protection against the effects of dextrin, and small doses of peptone and starch. On the contrary, the incidence of pulmonary hemorrhages was increased. Experimentally at least, the protective power of epinephrine in the proteose therapy of Nolf has not been confirmed.

Conclusions. When injected together with the antigen or immediately preceding it, epinephrine in the dosage of 0.0005 cc. of 1 : 10,000 per gram of body weight intravenously prevents death from true anaphylactic shock in guinea-pigs. The partial

protection afforded by epinephrine intravenously against the symptoms from arsphenamine disturbances is attributed to circulatory improvement. Epinephrine exerts no protection against the effects of agar sol, agar sol-gel, dextrin, peptone (small doses), starch, and when injected after or too long before the antigen in serum sensitized animals.

On the basis of the results obtained with atropin and epinephrine, the mechanism of action of agar and similar agents bears no relationship to true anaphylaxis or anaphylactic shock.

IV. CONCLUSIONS

1. The intravenous injection of atropine in guinea-pigs in the dosage of 0.01 mgm. per gram of body weight can completely prevent the toxic effects produced by the intravenous injection of beef serum; and peptone in doses corresponding to those used in the proteose therapy of Nolf. Partial protection was obtained against the effects (principally symptoms of respiratory distress) of agar sol, agar sol-gel, arsphenamine, congo red, peptone (larger doses), serum-sensitization (death in anaphylactic shock) and typhobacterin. No protection was obtained against the effects of acacia, althea, dextrin, pollen extract and starch.

2. When injected together with the antigen or immediately preceding it, epinephrine in the dosage of 0.0005 cc. of 1 : 10,000 per gram of body weight intravenously prevents death from true anaphylactic shock in guinea-pigs. The partial protection afforded by epinephrine (intravenously) against the symptoms of arsphenamine disturbances is attributed to circulatory improvement. Epinephrine exerts no protection against the effects of agar sol, agar sol-gel, dextrin, peptone (small doses), starch and when injected after or too long before the antigen in serum-sensitized animals.

On the basis of the results obtained with atropine and epinephrine, the mechanism of action of agar and similar agents bears no relationship to true anaphylaxis or anaphylactic shock.

REFERENCES

(1) Hanzlik and Karsner: J. Pharm. Exp. Therap., 1920, xiv, 379.
(2) Auer: Am. J. Physiol., 1910, xxvi, 439.
(3) Biedl and Kraus: Zent. f. Physiol., 1910, xxiv, 258.
(4) Baehr and Pick: Arch. f. exp. Path. Pharm., 1913, lxxiv, 65.
(5) Karsner and Nutt: J. Am. Med. Assoc., 1911, lvii, 1023.
(6) Januschke and Pollak: Arch. f. exp. Path. Pharm., 1911, lxvi, 205.
(7) Milian: Bull. Soc. francaise de dermat. et de syph., 1912, xxiii, 520; 1913, xxiv, 272.
(8) Beeson: Am. J. Syphilis, 1919, iii, 129.
(9) Hirano: Kitasato Arch. Exp. Med., 1919, lxxii, 241; Jap. Med. World, 1919, June 22.
(10) Stokes: J. Am. Med. Assoc., 1919, lxxii, 241.
(11) Nolf: J. Am. Med. Assoc., 1919, lxxii, 1901.
(12) Pelz and Jackson: J. Pharm. Exp. Therap., 1918, xi, 173.
(13) Novy and DeKruif: J. Inf. Dis., 1917, xx, 536.

EFFECTS OF VARIOUS COLLOIDS AND OTHER AGENTS WHICH PRODUCE ANAPHYLACTOID PHENOMENA ON BRONCHI OF PERFUSED LUNGS[1]

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I. INTRODUCTION

The results previously reported (1) with agar and similar colloids on untreated guinea-pigs and those treated with atropine and epinephrine (2) indicated no relationship between the pulmonary changes and effects that were produced and true anaphylaxis or anaphylactic shock. However, the pulmonary distension produced by agar is so marked, even after prophylactic treatment with atropine and epinephrine, that further proof is required in order to disprove definitely the analogy between this and anaphylactic shock. Moreover, objection might be raised against the too free interpretation of our results in intact animals. So far as bronchiolar effects are concerned this can be settled by perfusion of the surviving lungs. It is the object, therefore, of

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this paper to report the results with agar and other agents on bronchioles directly in surviving lungs. Arsphenamine was also tried in order to make the proof still more conclusive against this agent.

II. METHOD

The lungs of guinea-pigs were perfused according to the method of Baehr and Pick (3). All animals were previously rather deeply anesthetized with ether. A glass T-tube was tied into the trachea and one limb of this was joined to an artificial respiration apparatus registering about 30 strokes per minute. The chest was split open; the pericardium slit, the pulmonary artery freed and two silk ligatures carefully placed underneath it with a small aneurism needle. Traction with the ligature toward the operator was made so as to shut off the blood from the right ventricle momentarily, and at the same time a long glass cannula was tied in, pointing toward the lungs. The cannula was then quickly connected with normal saline (in Mariotte bottles suspended on a small perfusion rack) and the left ventricle was quickly transected to allow escape of the perfusion fluid. The artificial respiration which had been applied in the mean time was continued throughout the experiment, regulating the escape of air from the tracheal T-tube so as to permit complete collapse of the lungs during the expiratory phase.

Complete permanent distension of the lungs in the inspiratory phase means bronchoconstriction as from histamine, peptone, or serum with lungs of sensitized animals. However, care must be exercised in interpreting the failure to collapse from edema. When edema occurs the perfusion fluid as a rule begins to issue from the trachea and the lungs appear soggy and very wet.

If the operation has been successful, the blood is washed out of the lungs within a minute or two and the lungs continue to distend and collapse for an hour or more when edema occurs and the preparation must be discarded. On the other hand, if the blood is incompletely washed out, which always occurs if there is too much delay in the operation after the pulmonary artery has been incised, then c'ots remain in the pulmonary vessels and

capillaries and the result is a very prompt and marked distension simulating the action of histamine, peptone and serum-sensitized lungs. In such an event, the blood and clots are easily recognizable, which is not the case when the operation is entirely successful and the inflation occurs. A properly perfused lung which has become at the same time distended from an active bronchoconstrictor like histamine or peptone is fluffy, very pale and floats on ether. We always took special care to note the presence or absence of blood in our preparations microscopically, since this was of very great importance in properly interpreting the results with agar, which forms emboli and thrombi, the effect of which is the same as of blood clots, fibrin, etc. This will be referred to again later.

In our experiments ordinary saline (0.9 per cent NaCl) was used as the vehicle for the drugs, although Tyrode's or Ringer's solution is better for perfusion experiments. Most of the animals used weighed about 400 grams each.

The concentrations of the different agents corresponded to those occurring in the blood of intact animals as used in the experiments previously reported (1; 2) by us. In several instances, notably with acacia and arsphenamine, the concentrations corresponded to those occurring in human blood after therapeutic doses of these agents. The different agents were dissolved in normal saline and prepared in exactly the same way as used in our former experiments with intact animals. All the solutions were filtered through a quantitative filter paper (Whatman no. 40) before they were used for perfusion. For details of preparation, etc., the first paper (1) of this series should be consulted.

Certain of the lungs perfused with agar, acacia and peptone were also treated with atropine and papaverine to help ascertain if the distension, which occurred, was due to bronchoconstriction or not. The peptone constriction is known to be relieved by atropine and served as a control.

After the perfusion was completed, small portions of the lungs were placed at once into Zenker's fluid, later stained and sectioned in the usual way and examined microscopically for the

presence of blood, emboli, distension, etc. The importance of this was pointed out above. So far as distension is concerned, the presence of moderate or slight local distension is of no significance, since the lungs in these preparations are purposely rather strongly distended by artificial respiration and a moderate amount of air is apt to be retained even in collapsed lungs, particularly when edema is present. Therefore, gross pulmonary distension is of far greater significance.

III. DISCUSSION OF RESULTS

1. Agar and active bronchoconstrictors

The results with these agents are presented in table 1. Peptone and histamine and horse serum (in sensitized lung) produced marked distension. These agents are known to stimulate bronchial muscle directly and served as controls for the results with agar sol-gel (1 : 6). Of these histamine was most active, producing a marked pulmonary distension in forty-five seconds, while peptone, and serum in sensitized lung, required about four and two minutes, respectively. Microscopically, no emboli or blood were present with such active bronchoconstrictors as peptone and in the serum sensitized lung.

On the other hand, the lungs perfused with agar sol-gel, invariably showed the presence of agar emboli and there was a prompt stoppage of the perfusion flow through the lungs. The emboli were composed of clumps of agar in the pulmonary vessels and capillaries. In other respects the results with agar perfusion were precisely the same as with peptone and the serum-sensitized lung, namely, a very marked, prompt and pale gross distension of the lungs occurring in two to three minutes after the perfusion flow was started. The lungs were lighter than ether indicating presence of distension and absence of edema. Experiments 129b and 149b represent perfusions of one lung each of 2 preparations previously perfused with dextrin and acacia, respectively. The lungs continued to collapse with dextrin and acacia, but when agar sol-gel was perfused through the unligated lung of each preparation they promptly distended in the same way as pre-

viously untreated lungs, indicating conclusively that dextrin and acacia do not inflate, confirming other experiments with these agents to be described presently, and that agar does so rather peculiarly. Unfortunately, the particular microscopic sections reveal no agar emboli or thrombi. However, the perfusion flow stopped promptly and there is every reason to believe that emboli would have been found if sections had been more fortunately selected. Greater significance is attached to the presence of emboli in untreated lungs than in these two lungs previously perfused with dextrin and acacia. Moreover, in experiments 70, 126 and 147 sections from both lungs were made and, therefore, the emboli were more adequately revealed.

TABLE 1

Perfusion of lungs with agar, peptone, histamine and horse serum

NUMBER OF EXPERIMENT	COMPLETE AND PERMANENT GROSS PULMONARY DISTENSION IN (MINUTES)	MICROSCOPIC EXAMINATION OF PERFUSED LUNG†
	Agar sol gel (1:6)	
	minutes	
70	3	Agar emboli; marked distension
126	3	Masses of agar in large vessels; moderate distension
129b	3	Moderate local distension; alveoli normal
147	2	Many agar emboli; marked distension
149b	2	Local distension; alveoli normal
	Peptone	
136	3½ (0.03 per cent peptone)	Marked distension only
D*	4 (1 per cent peptone)	" " "
	Horse serum (2.8 per cent; lung of sensitized animal)	
135	2	Moderate local distension; alveoli normal (Gross distension very marked)
	Histamine (1:100,000)	
D*	¾	(Gross distension very marked)

* Class demonstration experiment.

† All of the lungs in this table floated on ether and edema was absent.

These results indicate definitely that the mechanism of agar inflation is peripheral, local and probably mechanical owing to the presence of the emboli. However, the results thus far do not as yet constitute absolute conclusive proof against plain muscle stimulation as a factor coöperating with the emboli in reducing the calibre of the bronchi. That is, without the microscopic evidences of agar emboli, it would be impossible to differentiate the results of the experiments with agar from those with peptone and histamine, and the interpretation would be pulmonary distension from bronchoconstriction due to direct stimulation of bronchial muscle or the parasympathetic endings. This can be definitely resolved by means of atropine and papaverine.

2. *Effects of atropine and papaverine*

Tonically constricted bronchial musculature as from peptone, in sensitized lungs perfused with serum (anaphylactic), etc., can be relaxed when the lung preparation is perfused with atropine. Even histamine, perhaps the most powerful bronchoconstrictor known, can be antagonized by atropine (Baehr and Pick (3)), although this is difficult with low concentrations. In our experiments, high concentrations of atropine, namely 0.05 per cent and 0.1 per cent, were used.

The treatment with atropine was carried out in two ways: (1) in one set of experiments the lungs were perfused after inflation occurred, and (2) in another set of experiments preliminary perfusion of the lungs was carried out. Agar, acacia and peptone were studied in this way with atropine and agar with papaverine. The results are presented in table 2.

These indicate that atropine has no effect on the pulmonary distension produced by agar sol-gel (1 : 6). Distension occurred promptly, that is, in three minutes, and the pulmonary vessels were loaded with agar emboli in the absence of blood in the same way as unatropinized lungs. On the other hand, the effects from peptone, which is a parasympathetic stimulant, were antagonized by atropine. This shows that the inflation by agar is not due to bronchoconstriction from stimulation of parasympathetic endings.

TABLE 2

Effect of atropine and papaverine on lungs perfused with agar, acacia and peptone

NUMBER OF EXPERIMENT	AGENT	CHANGES IN PERFUSED LUNG	MICROSCOPIC EXAMINATION OF PERFUSED LUNG AND REMARKS
	Atropine sulphate (0.1 per cent) after perfusion with agent		
126	Agar sol gel (1:6)	Distended in 3 minutes; no collapsing in 5 minutes after atropine	Masses of agar in large vessels; moderate distension; lungs floated on ether
128	Acacia (2 per cent)	Partial distention in 9 minutes; no collapsing in 9 minutes after atropine	Fibrin thrombi in large vessels; lungs floated on ether
136	Peptone (0.03 per cent)	Distension in 4 minutes; some collapsing in 10 minutes after atropine	Marked distension; lungs floated on ether
	Preliminary perfusion with atropine sulphate		
144	Agar sol gel (1:6)	Atropine (0.1 per cent) perfused for 4 minutes; complete distension in 3 minutes after agar was started	Agar emboli in large vessels; moderate distension; lungs floated on ether
148	Agar sol gel (1:6)	Atropine (0.1 per cent) perfused for 5 minutes; complete marked distension in 2 minutes after agar was started	Many agar emboli; moderate distension; lungs floated on ether
145	Acacia (1.7 per cent)	Atropine (0.1 per cent) perfused for 5 minutes; collapsed well for 20 minutes after acacia was started	Slight local distension; lungs submerged in ether
150	Peptone (0.05 per cent)	Atropine (0.05 per cent) perfused for 5 minutes before peptone was started; collapsed well for 20 minutes	Slight local distension; alveoli normal; lungs sank in ether
	Papaverine HCl (0.1 per cent)		
163	Agar sol gel (1:6)	Papaverin perfused for 5 minutes; marked distension in 3 minutes after agar was started; perfusion flow stopped	Lungs floated on ether; moderate local distension; agar thrombi in larger vessels; no bronchoconstriction

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	AGENT	CHANGES IN PERFUSED LUNG	MICROSCOPIC EXAMINATION OF PERFUSED LUNG AND REMARKS
		Papaverine HCl (0.1 per cent)—*Continued*	
164	Agar sol gel (1:6)	Papaverine perfused for 6 minutes; partial distension in 6 minutes, complete and marked distension in 9 minutes after agar was started	Lungs floated on ether; moderate local distension; agar thrombi in larger vessels; no bronchoconstriction
165	Agar sol gel (1:6)	Papaverine perfused for 4 minutes before, then papaverine together with agar for 5 minutes, when perfusion flow stopped and lungs were markedly and completely distended	Lungs floated on ether; marked distension (local in one section, general in the other); no thrombi

The results with papaverine were also negative, indicating definitely that the inflation from agar is not due to stimulation of bronchial muscle itself. That agar is not a smooth muscle stimulant will be further supported by the negative results obtained with surviving intestine and uterus to be described in a subsequent paper.

These results prove conclusively that inflation of lungs by agar is entirely peripheral, local and mechanical in the absence of active bronchial stimulation. This is also the main mechanism in the agar inflation of intact animals. The greatest significance is attached to the presence of agar emboli in perfused lungs, and thrombi of the pulmonary vessels in intact animals. Bearing in mind this and the results of our analysis, the mechanism of agar action appears to be as follows.

Mechanism of agar action. The agar forms thrombi, which block the small pulmonary vessels, surrounding the bronchioles, which in turn are compressed by the *vis a tergo* of the pulmonary flow from the right heart of the intact animal, and of the perfusion fluid in surviving lungs. In the perfused lung the flow of perfusion fluid stops. The compressed bronchioles are suf-

ficiently narrowed in calibre to prevent the escape of the air forced in by the violent efforts of inspiratory dyspnea of the intact animal and the artificial respiration of the perfused lungs. In other words, there is a passive bronchoconstriction (really a compression of the bronchi), and as a result permanent inflation of the lung. Since there is no increased excitability of parasympathetic endings or bronchial muscle, it is obvious why atropine and papaverine are ineffective in perfused lungs, and atropine and epinephrine in intact animals. That the effect is purely peripheral, local and mechanical is attested to by the results with lung perfusion, and, on this account the conclusion is forced that this is the principal and primary mechanism of agar action in intact animals. Although, of course, as a result of the asphyxia which supervenes after the pulmonary inflation develops and respiratory gaseous interchange is interfered with, it is conceivable that additional bronchoconstriction is superimposed as a result of central asphyxial stimulation of the vagus (parasympathetic) on the passive constriction primarily brought about. Asphyxial stimulation is probably the principal mechanism with other agents (colloids and others) that do not effectively occlude the pulmonary vessels of intact and perfused lungs, and in which circulatory disturbances play the leading rôle. However, with agar, we believe that passive bronchoconstriction by compression from emboli and thrombi as explained above is the primary determining mechanism of the pulmonary distension and subsequent phenomena.

3. Inactive agents

The results presented in table 3 indicate that the following agents; acacia, althea, arsphenamine, congo red, dextrin, gelatin, glycogen, nuclein solution and soluble starch, do not distend perfused surviving lungs. Therefore, they do not cause bronchoconstriction peripherally. The pulmonary inflations which occurred in intact animals, even though relatively moderate and inconstant, with the following; acacia, dextrin, althea, starch, congo red, and gelatin, might be of central origin as a result of

TABLE 3

Perfusion of lungs with various colloids and arsphenamine

NUMBER OF EXPERIMENT	GROSS PULMONARY DISTENSION ABSENT END OF	MICROSCOPIC EXAMINATION OF PERFUSED LUNGS	REMARKS
		Acacia (1.7 per cent)	
	minutes		
127	10		Lungs floated on ether
127	20 (distended)	Marked distension	Lungs floated on ether; edematous
128	9 (partial distension)	Marked distension	Fibrin thrombi in few veins; 2 per cent acacia used Lungs floated on ether; edema present
146	15	Distended in some areas only	Lungs sink in ether; edema marked
149a	14	Moderate local distension; alveoli normal	Lungs floated on ether
		Althea (2.5 per cent)	
130	9	Marked distension	Lungs sank in ether; very edematous
131	20	Moderate local distension; alveoli normal	Edema present
		Arsphenamine (0.01 and 0.02 per cent)	
139	17	Marked distension in local areas; alveoli normal; golden brown masses size of erythrocytes and others smaller, resembling hemosiderin granules	0.01 per cent arsphenamine used; lungs floated on ether
140	15	Irregular distension; no pigment	0.02 per cent arsphenamine used; marked edema present
		Congo red (0.17 per cent)	
132	15	Slight local distension; alveoli normal; conglutination thrombi in numerous capillaries; corpuscles stained orange; no blood in larger vessels	Lungs sank in ether; marked edema
133	18	Same as in Experiment 132	Marked edema present

TABLE 3—*Continued*

NUMBER OF EXPERIMENT	GROSS PULMONARY DISTENSION ABSENT END OF	MICROSCOPIC EXAMINATION OF PERFUSED LUNGS	REMARKS
		Dextrin (1 per cent)	
	minutes		
129a	22	Moderate local distension; alveoli normal	Lungs floated on ether
138	18	Marked distension; alveoli normal	Lungs hemorrhagic; float on ether
		Gelatin (1 per cent)	
134 a	16	Marked distension	Lungs floated on ether; edema present
		Glycogen (0.1 per cent)	
137	16	Marked distension; alveoli normal	Actual concentration of glycogen = 0.083 per cent, lungs sank in ether; edema present
163	15	Marked distension	Edema present
		Nuclein solution (0.1 per cent)	
143	20	Marked distension	Lungs submerge in ether; marked edema
		Soluble starch (1 per cent)	
141	15	Few areas of local distension; alveoli normal	Lung sinks in ether; some edema
142	16	Areas of distension more numerous; alveoli normal	Lung floats on ether; edema present

circulatory injury, which accompanies the effects, and due to asphyxia. So far as perfused lungs are concerned, emboli were not demonstrable with althea, congo red, dextrin, gelatin, glycogen, nuclein solution and starch, and thrombi were also absent in intact animals.

Acacia produced distension in two out of five lungs that were perfused although this was not prompt and one of the lungs was found to contain fibrin (blood) thrombi on microscopic exami-

nation which is sufficient to account for the inflation. Bronchoconstriction was finally excluded by means of atropine (see table 2). The majority of animals injected with acacia intravenously showed the presence of pulmonary thrombi, but the perfused lungs were uniformly negative as to this. Therefore, their occurrence in the circulation of the intact animal must be of greater significance. There is no reason to doubt that these would not act like agar thrombi and produce a passive bronchoconstriction, although the effects may well be mixed with asphyxia from circulatory injury arising primarily or together with the blocking of the pulmonary vessels. In another paper we shall submit evidence that acacia agglutinates blood corpuscles *in vitro*. This would tend to support the mechanism of thrombi formation *in vivo* with its consequent deleterious effects.

Concerning arsphenamine it can be said that this was entirely negative as to lung inflation, and, therefore, peripheral bronchoconstriction is excluded. It was previously shown that pulmonary inflation was also absent in intact animals. All of these results taken together prove conclusively that the disturbances from the intravenous injection of arsphenamine bear no relationship whatsoever to anaphylactic shock or anaphylaxis.

IV. CONCLUSIONS

1. Perfusion of the lungs of guinea-pigs with agar sol gel (1:6) causes prompt and marked distension of the lungs and stoppage of the perfusion flow due to the massing of agar emboli in the pulmonary vessels.
2. Prompt distension of perfused lungs occurs with peptone and histamine, but in the absence of pulmonary emboli, and this is due to active stimulation of bronchial muscle.
3. The distension from agar is not due to direct stimulation of bronchial musculature or parasympathetic endings, since the effects are not antagonized by papaverine and atropine.
4. The mechanism of agar action consists of a passive bronchoconstriction by compression of bronchioles from emboli in the pulmonary vessels.

5. Passive bronchoconstriction is also the chief mechanism in the marked pulmonary inflation produced by agar in intact guinea-pigs, whose pulmonary vessels contain thrombi invariably, although, of course, a superimposed bronchial stimulation from central stimulation occurring as a result of the supervening asphyxia is conceivable as a secondary phenomenon.

6. The following agents produced no gross distension of perfused lungs, therefore, no peripheral bronchoconstriction; acacia, althea, arsphenamine, congo red, dextrin, gelatin, glycogen, nuclein solution and soluble starch. Whatever inflation these agents produce (though variably) in intact animals, appears to be of central origin due to asphyxia arising from circulatory injury, which exists. When the inflation is absent the effects are entirely circulatory (arsphenamine).

REFERENCES

(1) Hanzlik and Karsner: J. Pharm. Exp. Therap., 1920, xiv, 379.
(2) Hanzlik and Karsner: J. Pharm. Exp. Therap., 1920, xiv, 425.
(3) Baehr and Pick: Arch. f. exp. Path. Pharm., 1913, lxxiv, 65.

EFFECTS OF VARIOUS COLLOIDS AND OTHER AGENTS WHICH PRODUCE ANAPHYLACTOID PHENOMENA ON SURVIVING INTESTINE AND UTERUS[1]

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OBJECT

All the evidences that have been thus far obtained by us (1, 2, 3) indicate that the marked inflation of the intact and surviving lungs, produced by agar is not due to direct stimulation of bronchial muscle or parasympathetic endings. The effects of various other non-protein colloids are variable, though quite similar with some, notably acacia. The mechanism of action of these agents was discussed in a previous communication. However, in order to make the proof against smooth muscle stimulation by these agents still more conclusive, experiments have been made on surviving intestine and uterus.

METHODS

The surviving intestine (longitudinal and circular strips) of rabbits and guinea-pigs and longitudinal strips of rabbit's virgin uterus were used. The strips were conveniently attached to weighted levers, which recorded the movements of the organs on a slow moving kymograph. For immersion, 50 cc. of Ringer's solution (mammalian) was used in tall glass cylinders resting in a large water bath maintained at 38°C. The various agents were added after peristalsis was fairly constant.

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TABLE 1

Effects of peptone, serums and various non-protein colloids on surviving intestine

NUMBER OF EXPERIMENT	AGENT	END CONCENTRATION IN RINGER'S SOLUTION	SPECIES	EFFECT ON PERISTALSIS	KIND OF STRIP
Peptone (10 per cent)					
		per cent			
165	Peptone	0.01	Rabbit	Slight stimulation; increased amplitude	Circular
	Peptone	0.03	Rabbit	Slight stimulation; increased amplitude	Circular
	Peptone	0.2	Rabbit	Marked stimulation; increased amplitude	Circular
	Peptone	1.0	Rabbit	Moderate stimulation; increased amplitude	Circular
165	Peptone	0.03	Rabbit	None	Longitudinal
	after acacia	0.2			
	after acacia	2.0	Rabbit	None	Longitudinal
	Peptone	0.2	Rabbit	Moderate stimulation	Longitudinal
	after acacia	0.2			
165	Peptone	0.2	Rabbit	Gradual stimulation, increased amplitude	Longitudinal
	after rabbit's serum	2.0			
166	Peptone	0.2	Rabbit	Mild stimulation	Longitudinal
	after agar	0.05			
	Peptone	0.03	Rabbit	None	Longitudinal
	after agar	0.05	Rabbit		
	Peptone	0.03	Rabbit	None	Longitudinal
	after dextrin	1.2			
Acacia (6 per cent)					
165	Acacia	0.1	Rabbit	Depression with prompt recovery; lessened amplitude	Longitudinal
165	Acacia	1.0	Rabbit	Marked depression of amplitude; gradual recovery	Longitudinal

166	Acacia	1.0	Rabbit	Moderate depression of amplitude; partial recovery	Longitudinal
166	Acacia	1.0	Rabbit	Abolition of peristalsis, complete recovery on removal of acacia	Longitudinal
165	Acacia after Congo red	1.0 0.02	Rabbit	Marked depression of amplitude; gradual recovery	Longitudinal
		Dextrin (6 per cent)			
165	Dextrin	0.6	Rabbit	Moderate depression; lessened amplitude and rate	Longitudinal
165	Dextrin	0.6	Rabbit	Gradual abolition of peristalsis, slow recovery	Longitudinal
166	Dextrin	1.2	Rabbit	Moderate depression of amplitude; no recovery	Longitudinal
		Glyeogen (2.5 per cent)			
165	Glycogen	0.1	Rabbit	Practically no effect	Longitudinal
165	Glycogen	0.1	Rabbit	None	Longitudinal
166	Glycogen	0.1	Rabbit	None	Longitudinal
166	Glycogen	0.1	Rabbit	None	Longitudinal
		Gelatin (6 per cent)			
165	Gelatin	1.2	Rabbit	Marked depression in rate and amplitude, slow, gradual recovery	Longitudinal
166	Gelatin	1.2	Rabbit	Moderate depression; rapid recovery	
166	Gelatin	1.2	Rabbit	Marked prompt depression of rate and amplitude; gradual recovery	Longitudinal
166	Gelatin	1.2	Rabbit	Abolition of peristalsis	Longitudinal

TABLE 1—*Continued*

NUMBER OF EXPERIMENT	AGENT	END CONCENTRATION IN RINGER'S SOLUTION	SPECIES	EFFECT ON PERISTALSIS	KIND OF STRIP
			Soluble starch (6 per cent)		
		per cent			
165	Starch	1.2	Rabbit	Moderate stimulation; increase in amplitude gradual	Longitudinal
166	Starch	1.2	Rabbit	Momentary insignificant depression of amplitude	Longitudinal
			Serums (whole)		
165	Human (old; preserved with 0.3 per cent trikresol)	0.4	Rabbit	Mild transitory depression of amplitude	Longitudinal
165	Human (old; preserved with 0.3 per cent trikresol)	2.0	Rabbit	More prolonged depression of amplitude	Longitudinal
165	Human (old; preserved with 0.3 per cent trikresol)	2.0	Rabbit	More prolonged depression of amplitude	Longitudinal
166	Human (old; preserved with 0.3 per cent trikresol)	4.0	Rabbit	Abolished peristalsis for short period, gradual recovery	Longitudinal
165	Rabbit serum (old); undecomposed	2.0	Rabbit	Moderate stimulation; increased amplitude	Longitudinal
165	Rabbit serum (old); undecomposed	2.0	Rabbit	Stimulation; increased amplitude	Longitudinal
165	Rabbit serum (fresh)	4.0	Rabbit	Stimulation moderate	Longitudinal
166	Rabbit serum (old; preserved with trikresol)	4.0	Rabbit	Almost total abolition of peristalsis, recovery on removal of serum	Longitudinal
165	Horse serum (old; preserved with trikresol)	2.0	Rabbit	Depression of peristalsis (decreased amplitude)	Longitudinal

165	Beef serum (old; preserved with trikresol)	2.0	Rabbit	Abolition of peristalsis	Longitudinal
166	Beef serum (plain; old) undecomposed	2.0	Rabbit	Mild stimulation; increased tone and rate	Longitudinal
166	Beef serum (plain; old) undecomposed	4.0	Rabbit	Marked stimulation; increased tone rate and amplitude somewhat	Longitudinal
				Congo red (1 per cent)	
165	Congo red	0.2	Rabbit	Marked stimulation; increased tone and amplitude	Longitudinal
165	Congo red	0.02	Rabbit	Moderate lasting stimulation	Longitudinal
165	Congo red	0.02	Rabbit	Considerable stimulation (chiefly increased amplitude)	Longitudinal
166	Congo red	0.08	Rabbit	Marked stimulation (increased tone and amplitude)	Longitudinal
166	Congo red	0.2	Rabbit	Definite stimulation (increased amplitude)	Longitudinal
166	Congo red	0.02	Rabbit	Moderate stimulation (increased amplitude)	Longitudinal
				Nuclein solution (Abbott)	
165	Nuclein solution (fresh)	2.0	Rabbit	Momentary abolition of peristalsis with gradual and prolonged recovery	Longitudinal
166	Nuclein solution (fresh)	2.0	Rabbit	Lasting continual depression (lessened tone); recovery on removal of nuclein	Longitudinal

TABLE 1—*Continued*

NUMBER OF EXPERIMENT	AGENT	END CONCENTRATION IN RINGER'S SOLUTION	SPECIES	EFFECT ON PERISTALSIS	KIND OF STRIP
		Althea extract (15 per cent)			
165	Althea extract (slightly acid)	0.3	Rabbit	Gradual depression (lessened amplitude)	Longitudinal
165	Althea extract (slightly alkaline)	0.3	Rabbit	None	Longitudinal
166	Althea extract (slightly acid; natural)	2.0	Rabbit	Abolition of peristalsis; recovery on removal of althea	Longitudinal
166	Althea extract (slightly acid; natural)	0.2	Rabbit	Gradual depression and abolition (lessened amplitude) recovery on removal of althea	Longitudinal
166	Althea extract (neutral)	0.2	Rabbit	Momentary depression followed by stimulation	Longitudinal
166	Althea extract (neutral)	1.0	Rabbit	Marked lasting stimulation (increased tone and amplitude)	Longitudinal
166	Althea extract (neutral)	1.0	Rabbit	Momentary depression, followed by considerable lasting stimulation (increased amplitude)	Longitudinal
166	Althea extract (neutral)	2.0	Rabbit	Marked prompt stimulation (increased tone and amplitude)	Longitudinal
	Althea extract (neutral)	1.0	Rabbit	Prompt moderate stimulation (increased tone and amplitude)	Longitudinal
166	Althea extract (neutral)	1.0	Rabbit	Prompt moderate stimulation (increased tone and amplitude	Longitudinal
166	Althea extract (very slightly acid; natural)	1.0	Rabbit	Gradual temporary depression (lessened tone and amplitude)	Longitudinal

166	Althea extract (very slightly acid; natural)	2.0	Rabbit	Moderate gradual, lasting stimulation (increased amplitude)	Longitudinal
	Althea extract (very slightly acid; natural)	1.0	Rabbit	Moderate stimulation (lessened tone, increased amplitude)	Longitudinal
Agar sol (0.5 per cent) and agar sol gel (1 : 6)					
166	Agar sol	0.02	Rabbit	None	Longitudinal
166	Agar sol	0.05	Rabbit	None	Longitudinal
166	Agar sol	0.10	Rabbit	Slight depression (slowed rate)	Longitudinal
70	Agar sol	0.01	Guinea-pig	None	Longitudinal
70	Agar sol	0.05	Guinea-pig	Slight depression (slowed rate)	Longitudinal
73	Agar sol	0.02	Guinea-pig	Slight depression (slowed rate)	Longitudinal
73	Agar sol gel	0.017	Guinea-pig	None	Longitudinal
73	Agar sol gel	0.017	Guinea-pig	None	Longitudinal
73	Agar sol gel	0.017	Guinea-pig	None	Circular

TABLE 2

Effects of peptone, serum and various non-protein colloids on surviving uterus of rabbit (longitudinal strips)

NUMBER OF EXPERIMENT	AGENT	END CONCENTRATION IN RINGER'S SOLUTION	EFFECT ON PERISTALSIS
	Peptone (10 per cent)		
		per cent	
165	Peptone	0.2	Stimulation (increased rate and amplitude)
165	Peptone	0.4	Further stimulation
167	Peptone	0.03	Moderate stimulation (increased amplitude and rate)
167	Peptone	1.0	Marked lasting stimulation (increased tone and rate at first; later, lessened, increased amplitude)
	Acacia (6 per cent)		
167	Acacia	1.0	Depression more lasting (lessened tone and amplitude)
167	Acacia	1.2	Prompt marked lasting depression; gradual recovery (chiefly lessened tone)
	Dextrin (6 per cent)		
167	Dextrin	1.2	Depression; gradual recovery (lessened tone and amplitude)
	Glycogen (2.5 per cent)		
165	Glycogen	0.15	Depression (lessened amplitude)
167	Glycogen	0.1	Depression (lessened tone and amplitude)
167	Glycogen	0.1	Moderate temporary depression

Gelatin (6 per cent)			
165	Gelatin	1.2	None
167	Gelatin	0.48	Marked temporary depression (lessened tone and amplitude)
167	Gelatin	1.2	Prompt lasting marked depression; (lessened tone and amplitude); gradual recovery
Soluble starch (6 per cent)			
165	Starch	1.2	None
167	Starch	1.2	Gradual temporary depression (lessened tone and amplitude)
Serums (whole)			
165	Human (old, preserved with trikresol)	4.0	None
167	Human (old, preserved with trikresol)	8.0	Gradual depression (lessened tone); recovery on removal of serum
167	Human (old; preserved with toluol removed)	2.0	Gradual depression (lessened tone); recovery on removal of serum
167	Rabbit (old; preserved with trikresol)	2.0	Marked depression; no recovery, (lessened tone and amplitude)
167	Rabbit (old; preserved with trikresol)	2.0	Further depression
167	Rabbit (old; dialyzed; undecomposed)	2.0	Considerable lasting stimulation (increased tone, amplitude and rate)
165	Beef (old; preserved with trikresol)	4.0	None
167	Beef (old; no preservative; undecomposed)	2.0	Stimulation (prevention of falling tone; diminished amplitude)
167	Beef dialyzed)	2.0	Depression (loss of tone and amplitude); recovery on removal

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	AGENT	END CONCENTRATION IN RINGER'S SOLUTION	EFFECT ON PERISTALSIS
	Congo red (1 per cent)		
165	Congo red	0.04	Very slight stimulation (increased amplitude)
167	Congo red	0.04	Considerable stimulation (increased tone and amplitude)
167	Congo red	0.2	Lasting stimulation (increased tone)
	Nuclein solution (Abbott)		
165	Nuclein solution	3.0	None
165	Nuclein solution	1.0	Considerable depression (lessened tone and amplitude)
165	Nuclein solution	2.0	Considerable depression (gradual recovery)
	Althea extract (10 per cent and 15 per cent)		
165	Althea extract (15 per cent; slightly acid)	1.5	Depression
167	Althea extract (10 per cent; neutral)	2.0	Stimulation (gradual increase in tone and amplitude)
167	Althea extract (10 per cent; slightly acid; natural)	2.0	Marked depression and temporary abolition of peristalsis (lessened tone, and amplitude; gradual recovery)
	Agar (0.5 per cent)		
165	Agar sol	0.02	None
165	Agar sol	0.02	None
167	Agar sol	0.1	Temporary depression; prompt recovery (lessened amplitude)

	Pollen extract (0.01 mgm. nitrogen to 1 cc.; Mulford's)		
167	Hay fever spring	2.0	Depression (lessened tone)
167	Hay fever fall	2.0	Depression (lessened tone)
	Trikresol (0.3 per cent)		
167	Trikresol	0.006	Prompt marked depression (lessened tone); gradual recovery; complete recovery on removal of trikresol

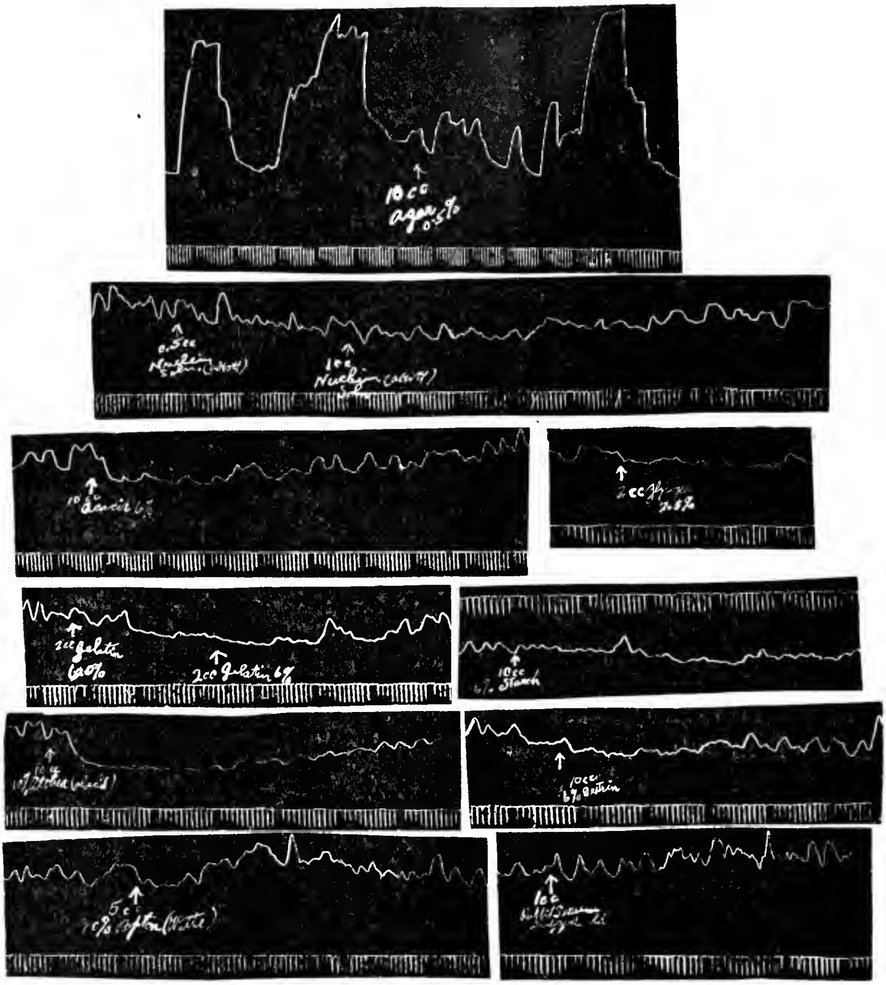

FIG. 1. EXPERIMENT 167

Showing effects of agar (1 per cent), acacia (1.7 per cent), starch (1.2 per cent), glycogen (0.1 per cent), althea (2 per cent), dextrin (1.2 per cent), gelatin (1.2 per cent), nuclein solution (1 cc. = 0.02 per cent), peptone (1 per cent), and rabbit's serum (0.2 per cent) on surviving rabbit's uterus in Ringer's solution (50 cc.). The figures in parentheses denote end concentrations. Time: each stroke = 5 seconds. Depression was produced by all agents except peptone and rabbit's serum which acted as controls and stimulated uterine persistalsis.

The concentrations of the different agents varied. These ranged from concentrations occurring in blood after the intravenous injection of these in the experiments on systemic actions previously reported (1, 2) up to very high concentrations in order to see if augmentor effects could be obtained.

None of the animals had been previously treated with any of the agents used. The experiments on rabbit's uterus agreed so closely with those on the intestine of both rabbit and guinea-pig that it was deemed unnecessary to carry out additional experiments with guinea-pig's uterus.

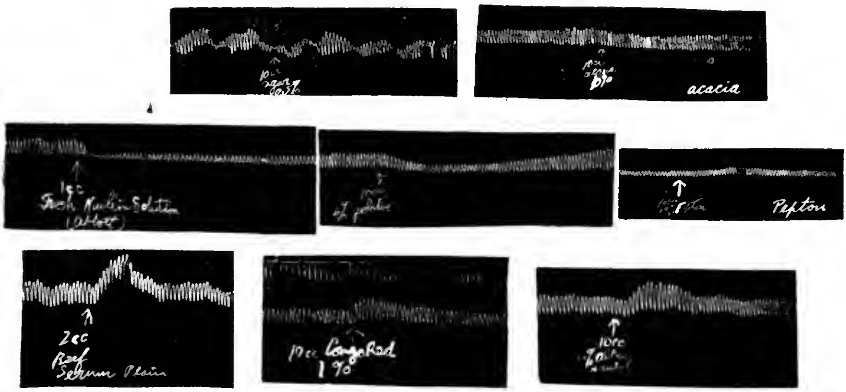

FIG. 2. EXPERIMENT 166

Showing effects of agar (1 per cent), acacia (2 per cent), nuclein solution (0.02 per cent), congo red (0.2 per cent), peptone (0.2 per cent), beef serum (4 per cent), and neutral althea (2 per cent) on longitudinal strip of rabbit's intestine in Ringer's solution (50 cc.). The figures in parentheses denote end concentrations. Depression was produced by agar, acacia, nuclein solution, and gelatin; stimulation by peptone, beef serum, congo red and neutral altrea.

FIG. 3. EXPERIMENT 165

Showing effects of rabbit's serum (0.2 per cent), horse serum (0.2 per cent), and glycogen (0.1 per cent) on longitudinal strip of rabbit's intestine in Ringer's solution (50 cc.). The figures in parentheses denote end concentrations of the different agents. Depression was produced by horse serum and glycogen; stimulation by rabbit's serum.

RESULTS

The results with surviving intestine are presented in table 1, and those with uterus in table 2. These indicate also the various concentrations that were used and conditions under which the experiments were performed. In addition, three figures, illustrating typical effects of the more important agents on the intestine and uterus, are included.

The results (as indicated by the data in the tables, and the figures) are so uniform and striking that further interpretation is practically unnecessary.

SUMMARY

The main results may be stated as follows:

Agar produces no effects on or slight depression of the peristalsis of surviving intestine and uterus. The following agents uniformly depressed both the intestine and uterus; acacia, dextrin, glycogen, gelatin, starch, human and horse serums and nuclein solution (Abbott). Althea extract was variable, although depression was the rule, no matter whether slightly acid, alkaline or neutral in reaction. The following agents produced moderate to marked stimulation of both uterine and intestinal peristalsis; peptone, congo red and rabbit's serum. Beef serum was irregular. So far as peptone and rabbit's serum are concerned the results obtained are confirmative of the usual effects, and these agents served as controls for the inactive agents, indicating that the organs were functionally active and responsive in the usual way when tested against well known augmentors of peristalsis. The results with congo red do not agree with those on surviving lungs in which inflation (bronchoconstriction) did not occur. However, this is immaterial to the results of this investigation.

So far as agar, acacia and the remaining non-protein colloids are concerned, the results confirm those obtained on surviving lungs. That is, these agents are not stimulants of plain muscle in the bronchi, uterus and intestine. On the contrary, they almost invariably depressed the intestinal and uterine muscle.

This would be generally expected with colloids, that is, a limitation of physiological activity because of their peculiar physical chemical properties (lessened diffusion and dissociation of ions, adsorption, etc.) with effects on nutrition.

As far as acacia is concerned, my results are confirmative of a preliminary report by Kruse (4). Accordingly, the use of acacia intravenously can not be regarded without the possibilities of causing injury, or at least effects that are not necessarily beneficial.

The results reported in this paper are in line with those on bronchial musculature described in previous papers, and prove conclusively that the disturbances produced by the intravenous injection of these and certain other agents such as arsphenamine, bear no relationship whatsoever to anaphylaxis or anaphylactic shock.

CONCLUSIONS

1. The direct application of agar sol and agar sol gel to surviving intestine and uterus produces either no effect or slight depression of peristalsis.

2. The following agents in high and low concentrations uniformly depressed the peristalsis of surviving intestine and uterus; acacia, dextrin, glycogen, gelatin, starch, human and horse serums and nuclein solution. Althea extract was somewhat variable, although depression was most common, irrespective of the chemical reaction.

3. The following agents used as controls produced moderate to marked stimulation of intestinal and uterine peristalsis: peptone, and rabbit's serum. Beef serum was irregular. Definite and rather marked stimulation was produced by congo red in low concentrations. This does not agree with the results obtained on the bronchi of perfused lungs.

4. These effects on intestinal and uterine musculature agree with those on bronchial musculature (except Congo red) previously reported.

5. The results of this study sustain the contention elaborated in previous papers as to bronchial musculature, that the disturbances produced by the intravenous injection of agar and various non-protein colloids, and also arsphenamine, bear no relationship whatsoever to anaphylaxis or anaphylactic shock.

REFERENCES

(1) Hanzlik and Karsner: J. Pharm. Exp. Therap. 1920, xiv, 379, 425.
(2) Hanzlik and Karsner: J. Pharm. Exp. Therap., 1920, xiv, 379, 425.
(3) Hanzlik and Karsner: J. Pharm. Exp. Therap., 1920, xiv, 449.
(4) Kruse: Am. J. Physiol., 1919, xlix, 137 (Proc.).

HEMAGGLUTINATION IN VITRO BY AGENTS WHICH PRODUCE ANAPHYLACTOID SYMPTOMS[1]

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INTRODUCTION

Certain of the colloid and other agents described in previous papers (1, 2) by us were found to produce pulmonary hemorrhages and thrombi in the pulmonary capillaries after intravenous injection in guinea-pigs. Among these agar and acacia were found to produce these phenomena most constantly. The marked pulmonary distension produced by agar is attributed to passive bronchoconstriction produced by the thrombi and emboli in both intact and perfused lungs. With acacia and the remaining agents the thrombi, which were found in intact lungs, did not occur in perfused surviving lungs; and distension of these was variable or absent. Hence, the mechanism of thrombus formation in intact lungs must be regarded as of considerable importance particularly with agents which are used intravenously for therapeutic purposes such as acacia, certain thromboplastic agents, arsphenamine, peptone, etc. Conglutination of the corpuscles is conceivable in the formation of these thrombi.

Agglutination of cat corpuscles by acacia in vitro has been observed by Bayliss (3), reported by Robertson (4) with cat but not human corpuscles, and by Kruse (5) with human, dog, cat and rabbit, but not with ox, frog and turtle corpuscles. Other agents that were studied by us besides acacia, namely, agar,

[1] This investigation was supported in part by a grant from the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.

thromboplastin, coagulen, arsenicals, etc., were found to produce pulmonary thrombi and emboli and even more severe anaphylactoid symptoms. It is reasonable to suppose that these might cause agglutination even more strikingly than acacia. It was, therefore, resolved to investigate the entire series of colloid and other agents used in our previous studies on anaphylactoid symptoms with the hope that the results might further elucidate the accidents of intravenous medication and the causation of thrombi where these have been observed, although, of course, agglutination in vitro does not necessarily mean that the same occurs in vivo.

METHOD

Fresh human, cat and guinea-pig red blood corpuscles were washed three times with salt solution (0.9 per cent NaCl usually, but 0.85 per cent in a few of the tests) and made up to a 5 per cent suspension of the cells. One cubic centimeter of blood suspension was mixed with 1 cc. of the agent to be tested so that the final blood suspension was 2.5 per cent. The agents tested were in such concentration that the final mixture in the test tubes produced the desired end concentrations. These are indicated in the tables. Readings were made after two hours in a water bath at 38°C., and again after twenty-two hours additional in the refrigerator. Record was made not only of agglutination but also of hemolysis and precipitation.

CONCENTRATION OF AGENTS

At least two concentrations in the majority of tests were used: (1) Very low concentrations such as occur after the intravenous adminstration of certain of the agents therapeutically in human individuals; (2) Higher, and also low concentrations, which were used in our previous experiments on guinea-pigs with agents causing anaphylactoid symptoms. These concentrations were calculated on the basis of blood being equivalent to 6 per cent of body weight, which was taken as 300 grams for the average guinea-pig in our former experiments. In the case of man 60 kgm. was taken as the average weight. The concentrations in

cat's blood were the same as in guinea-pig's and human blood.

In addition, the lowest effective concentrations of those agents, which proved to be agglutinators, were ascertained as closely as possible.

RESULTS

All of the results that were obtained with the 31 different agents tested are presented in tables 1, 2, 3, and 4. These are further correlated in Tables 5, 6, and 7 with the results, previously reported (1, 2), and pertaining to pulmonary thrombi and hemorrhages after the injection of the same agents in guinea-pigs.

AGGLUTINATION AND THROMBOSIS

In interpreting and attempting to correlate the production of agglutination in vitro and thrombosis in vivo there are certain points which must be given consideration. Thrombosis occasionally but rarely occurs after normal salt solution injections as seen in one of our controls. Conglutination of corpuscles may occur as the result of fixation or of decomposition of tissues before fixation. In the present series, the lung tissues were cut into blocks not more than 4 mm. thick, placed in carefully prepared Zenker fluid not more than five minutes after the death of the animal, using 2 or 3 blocks in 30 to 50 cc. fluid. At the end of twenty-four hours the blocks were washed in freely running water for twenty-four hours and the usual paraffin method followed. As nearly as possible the procedure was uniform throughout. Therefore, the series served as its own control, and conglutination occurred very rarely following the injection of agents which otherwise did not produce thrombosis, namely, beef serum, hemostatic serum, althea, pollen extract, phylacogen and peptone. In previous studies by one of us (H. T. K.) it was shown that conglutination is common following the injection of beef serum and of peptone. We feel that in the present series it is unwise to lay much stress on conglutination of erythrocytes unless the agent employed has produced definite thrombosis with platelet and fibrin deposition. Table 5 gives the results of intravenous

TABLE 1

*Agglutinating agents**

AGENT	END CONCENTRATION	HUMAN CELLS		CAT CELLS		GUINEA PIG CELLS	
		2 hours	24 hours	2 hours	24 hours	2 hours	24 hours
	per cent						
Acacia (Khardofan)	1.0 (2)	+	+	+	+	+	+
	0.8	−	−	−	−	−	−
	0.5	−	−	−	−	−	−
	0.1 (2)	−	−	−	−	−	−
	0.05 (2)	−	−	−	−	−	−
	0.016	−	−	−	−	−	−
Althea	1.2	+	+	+	+	+	+
	0.62	±	+	+	+	+	+
	0.31	+	+	+	+	+	+
	0.15	±	+	+	+	+	+
	0.08	+	+	+	+	+	+
	0.05	−	−	−	−	−	−
	0.01	−	−	−	−	−	−
	0.005	−	−	−	−	−	−
Agar	0.055	+	+	+	+	+	+
Agar sol gel†	0.002	+	+	+	+	+	+
	0.001	±	+	±	+	±	+
	0.001	+	−	+	−	+	−
	0.0005	−	−	−	−	−	−
	0.00025	−	−	−	−	−	−
	0.00012	−	−	−	−	−	−
Arsphenamine‡	0.166	+	+	+	CH	+	CH
	0.083	+	+	+	CH	+	+
	0.04	+	+	+	+	+	+
Atropine sulphate	0.03(2)	+	+	+	+	+	+
	0.015	+	+	+	+	+	+
	0.005	±	+	±	+	±	+
	0.002	−	+	−	±	+	+
Beef serum		−	+	−	+	−	+

TABLE 1—*Continued*

AGENT	END CONCENTRATION	HUMAN CELLS		CAT CELLS		GUINEA PIG CELLS	
		2 hours	24 hours	2 hours	24 hours	2 hours	24 hours
	per cent						
Collargol	0.17	+	+	+	+	+	+
	0.011	−	−	−	−	−	−
	0.011	+	+	+	+	+	+
	0.005	+	+	+	+	+	+
Gelatin	1.0	+	+	+	+	+	+
	0.5	+	+	+	+	±	+
	0.1 (2)	+	+	+	+	+	+
	0.05	+	+	+	+	+	+
	0.01	−	−	−	−	−	−
	0.005	−	−	−	−	−	−
Neoarsphenamine	0.025 (ppt.)	+	+	+	+	+	+
	0.025	+	+	+	+	+	+
	0.025	−	−	−	−	+	+
Nuclein	10.0 (2)	−	+	−	+	−	+
	5.0	−	+	−	+	−	+
	0.6	−	+	−	+	−	+
Soluble starch	1.0 (3)	+	+	+	+	+	+
	0.5	+	+	+	+	+	+
	0.5	+	+	−	+	−	+
	0.1	−	−	−	−	−	−
	0.05	−	−	−	−	−	−
	0.01	−	−	−	−	−	−
Thromboplastin (Squibb)	17.0	+	+	+	+	+	+
	17.0	−	−	−	−	−	+
	2.8	+	+	+	+	+	+
	2.8	−	+	−	+	−	−
Tragacanth	0.05	+	+	+	+	+	+
Salt solution (0.9 per cent NaCl; control)		−	−	−	−	−	−

* The various signs and abbreviations have meanings as follows: (+) = agglutination present; (−) = agglutination absent; CH = complete hemolysis; figures in parentheses denote number of tests.

† Prepared according to the method of Novy and DeKruif: J. Inf. Dis., 1917, xx, 629.

‡ From Dermatological Research Laboratories.

injection of agglutinating substances. The results as to thrombosis and conglutination were reported in a previous paper of this series.

Of these agents it will be seen that there appears to be a definite correlation between agglutination and thrombosis in the case of acacia, agar, arsphenamine, beef serum, collargol, and thromboplastin, or in one-half the agents which caused agglutination in vitro. Nevertheless such marked agglutinators as althea, gelatin and starch failed to produce thrombosis except for one

TABLE 2

Agents failing to produce agglutination

AGENT	PER CENT	AGENT
Coagulen (Ciba)	0.5	Peptone, 0.002 per cent
Coagulen (Ciba)	0.1	Pollen Extract ‡, Hay Fever Fall, 6 per cent (2)*
Congo Red	0.34	Pollen extract ‡, Hay Fever Spring, 6 per cent (2)
Congo Red	0.17	Serum, Dog, 11 per cent
Dextrin	1.0	Serum, Horse, 11 per cent
Epinephrine	0.000083	Serum, Human, 11 per cent
Glycogen	0.1	Serum, Rabbit, 11 per cent
Glycogen†	0.05	Sodium arsenate, 0.013 per cent
		Sodium chloride, 0.9 per cent
Kephalin†	0.017	Trikresol, 0.05 per cent
Peptone (Witte)	1.0	Venarsen,§ 0.26 per cent

* Figures in parenthesis indicate number of tests.
† Prepared from pig's brains according to the method of Howell.
§ Principal constituent is sodium cacodylate.
‡ Furnished by H. K. Mulford and Company, Philadelphia.

animal in the althea series which showed conglutination of corpuscles.

Atropine was used in conjunction with numerous agents and thrombosis appears in connection with agar (two animals), arsphenamine (two animals, one of which shows only conglutination), typhobacterin (one animal), pollen extract (one animal showing conglutination). Of these agents agar and arsphenamine were commonly associated with thrombus formation and it may safely be stated that in spite of the surprising and marked

agglutination in the test tube produced by atropine sulphate, large intravenous doses failed to produce thrombosis.

TABLE 3

*Agents producing hemolysis**

AGENT	END CONCENTRATION	HUMAN CELLS		CAT CELLS		GUINEA PIG CELLS	
		2 hours	24 hours	2 hours	24 hours	2 hours	24 hours
	per cent						
Acacia	1.0	–	–	–	–	–	P
	0.5	–	–	–	–	–	P
Arsphenamine	0.166	–	–	P	C	P	C
	0.083	S	P	P	C	P	P
Coagulen (Ciba)	0.5 (2)	–	P	–	P	–	P
	0.1	–	S	–	S	–	S
	0.1	–	–	–	S	–	P
Collargol	0.011 (2)	–	P	–	P	–	P
Gelatin	2.5	–	P	–	S	–	P
	1.0	–	–	–	–	–	–
Kephalin	0.05	–	S	–	P	–	P
	0.017	–	–	–	S	–	P
Nuclein solution	0.6	–	–	–	–	–	P
Serum, Dog		–	P	–	P	P	C
Thromboplastin (Squibb)	17.0 (2)	–	P	–	P	–	P
	2.8 (2)	–	P	–	P	–	P
Thromboplastin (Armour)	17.0	–	P	–	P	–	P
Salt solution (0.9 per cent NaCl)	(6)	–	–	–	–	–	–

* The signs and abbreviations in this table have meanings as follows: – = no hemolysis; S = slight hemolysis; P = partial hemolysis; C = complete hemolysis.

In this connection it should be remembered that the solutions of atropine (particularly the higher concentrations) were acid. This is also true of althea extract. The agglutination in vitro, at least with high concentrations, can be explained by this, but

when injected intravenously the acidity is quickly neutralized by the reserve alkali unless injected in very large quantities. The hydrogen ion concentration of the mixtures was not estimated. On the other hand, with such agents as agar, acacia, collargol,

TABLE 4

Agents producing dark brown discoloration of blood

AGENT	END CONCENTRATION	HUMAN CELLS		CAT CELLS		GUINEA PIG CELLS	
		2 hours	24 hours	2 hours	24 hours	2 hours	24 hours
	per cent						
Althea	2.5	+	+	+	+	+	+
Arsphenamine	0.166	+	+	+	+	+	+
	0.083	+	+	+	+	+	+
	0.04	−	−	+	+	+	+
Gelatin	2.5	−	−	−	−	+	+
	1.0	−	−	−	−	+	+
	1.0	+	+	+	+	+	+
	0.1	+	+	+	+	+	+
Neoarsphenamine	0.023 (ppt.)	+	+	+	+	+	+
	0.023	+	+	+	+	+	+
	0.023	−	−	+	+	+	+
Peptone	1.0	+	+	+	+	+	+
Soluble starch	1.0	−	+	−	+	−	+
	0.5	−	+	−	+	−	+
Trikresol	0.05	+	+	+	+	+	+
Salt solution		−	−	−	−	−	−

The signs and abbreviations have meanings as follows: + = discoloration present; − = discoloration absent; ppt. = precipitate.

gelatin, beef serum, neoarsphenamine and starch, whose chemical reaction is practically neutral, the mechanism responsible for the agglutination (in vitro) must be different. Arsphenamine solutions are, of course, slightly alkaline (also serum), and how this would affect agglutination we do not know.

Of the 15 agents which failed to produce agglutination in the test tube the following 5 (coagulen, kephalin, pollen extract, human serum and sodium arsenate) produced thrombosis in the animals according to the summary in table 6. The results of the injection experiments have been previously reported.

TABLE 5

AGGLUTINATING AGENTS	TOTAL NUMBER OF ANIMALS INJECTED	NUMBER OF ANIMALS SHOWING	
		Thrombosis	Conglutination
Acacia	10	3	3
Agar	·14	7	
Althea	3		1
Arsphenamine	6	2	2
Beef serum	2		1
Collargol	3	1	1
Gelatin	2		
Neoarsphenamine	3		
Nuclein	3		
Soluble starch	3		
Thromboplastin (Squibb)	5	1	2
Atropine sulphate (with other agents)	21	4	2

TABLE 6

NON-AGGLUTINATING AGENTS	NUMBER OF ANIMALS INJECTED	NUMBER OF ANIMALS SHOWING	
		Thrombosis	Conglutination
Coagulen	8	1	1
Kephalin	4	2	
Pollen extract	7		3
Human serum	1	1	
Sodium arsenate	2	1	

Thus it will be seen that only a small number of the agents which are non-agglutinators show thrombosis and only 3 of these (kephalin, human serum and sodium arsenate) show any important relationship.

Of the non-agglutinators, epinephrine deserves separate comment. It was used in 10 animals in conjunction with other agents

and in 4 animals there was thrombosis. Two of these 4 were injected with agglutinating and thrombus producing agents, namely, agar and arsphenamine. The other 2 were injected with peptone and with horse serum (anaphylaxis), both animals showing only conglutination of corpuscles. Hence epinephrine is included among those agents which produced neither agglutination in the test tube nor thrombosis in the animals.

As far as the results with agglutination in vitro are concerned, these are confirmative of those of Ono (6) for gelatin, acacia, tragacanth and althea, and of Kruse (5) for acacia. The concentrations used by Ono and Kruse are not known to us. The results obtained by us with gelatin and acacia indicate that they can agglutinate human corpuscles with concentrations corresponding to those occurring in the intravenous administration of these as therapeutic agents in traumatic shock, hemorrhage, etc. This taken together with the fact that acacia forms pulmonary thrombi in guinea-pigs indicates that the use of acacia intravenously should be contemplated with definite possibilities of causing injury.

Concerning the mechanism of action of the agglutinating agents, the evidences which we have are insufficient to permit an analysis of this. Most of the agents that acted as agglutinators are colloids. However, the physical properties of certain of these are too different to permit of any general classification. For instance, acacia and gelatin in the concentration of 10 to 12 grams per liter possess osmotic pressures of 72 and 6 (mm. Hg.) and molar weights of 2400 and 36,000 (Taylor, W. (7)), respectively, yet both acted as agglutinators; gelatin somewhat more effectively. Other colloidal agents (see table 6) again did not agglutinate. Finally atropine, acids, and other agents which behave like crystalloids, also agglutinate. A colloidal state, therefore, does not seem to be indispensable for agglutination. The relation is most likely to be sought in the electric charges of the colloids.

HEMOLYSIS AND HEMORRHAGE

From the data in table 3, it is seen that the most notable hemolyzing agent was arsphenamine, which affected cat and guinea-pig cells more markedly than human cells. The least active of this group were acacia, nuclein and gelatin, acacia and gelatin affecting particularly the guinea-pig cells. The others ranged as hemolyzing agents of only moderate activity. It is to be noted that none of the agents completely hemolyzed human blood and only a few of the other bloods employed. The conditions in the test tube were arranged with the primary idea of testing for agglutination and were not ideal for hemolysis. It is possible that the presence of complement might have made a difference in

TABLE 7

HEMOLYTIC AGENTS	TOTAL NUMBER OF ANIMALS INJECTED	PULMONARY HEMORRHAGE PRESENT IN
Acacia	10	3
Arsphenamine	6	6
Coagulen (Ciba)	8	3
Collargol	3	1
Gelatin	2	2
Kephalin	4	2
Nuclein solution (Abbott)	3	2
Thromboplastin (Squibb)	5	4

the completeness of hemolysis but it is unlikely that other important variations from the result shown would have occurred. Table 7 shows the attempt at correlation between test tube hemolysis and pulmonary hemorrhage.

Dog serum might have been included in the above table but for the fact that no animals were injected in this series. It is well known, however, that dog serum is toxic for guinea-pigs and produces hemorrhage in the lungs of injected animals. Table 2 shows that it produced hemolysis in all the bloods employed, particularly that of the guinea-pig.

The hemolytic agents which produced hemorrhages in all animals injected are arsphenamine (alkaline) and gelatin, both of which also produced discoloration of the blood. Thromboplastin

and nuclein solution produced hemorrhages in a high percentage of animals. The other four hemolytic agents, namely, acacia, coagulen, collargol, and kephalin, produced hemorrhages in only a relatively small percentage of the animals employed.

Thirteen other agents, namely, althea, agar, beef serum, neoarsphenamine, starch, congo red, dextrin, peptone, pollen extract, human serum, rabbit serum, sodium arsenate and venarsen, produced no hemolysis, but distinct hemorrhages, and four agents (glycogen, horse serum, trikresol and normal saline) produced neither hemolysis nor distinct hemorrhage.

BLOOD DISCOLORATION

The data in table 4 indicate that discoloration of the blood was most markedly displayed by arsphenamine, a property also exhibited in slightly less degree by neoarsphenamine. As with hemolysis, blood discoloration was somewhat less marked with human blood than with cat and guinea-pig blood.

To what extent the discoloration of the blood that was observed, was due to methemoglobin alone can not be said definitely without more definite analysis than by direct inspection alone. A part of the discoloration was no doubt due to the formation of acid and alkali hematins from the following agents which gave evidences of definite chemical reaction toward litmus; althea extract (acid) and arsphenamine (alkaline). The formation of acid and alkali hematins can not be invoked for the following agents which were practically neutral in reaction; neoarsphenamine, gelatin, peptone, starch and trikresol. The discoloration from these agents may be regarded as due to methemoglobin with greater certainty. Unfortunately, the hydrogen ion concentration of the different mixtures was not ascertained. The incidence of blood discoloration and pulmonary hemorrhage was too variable to warrant the drawing of definite conclusions.

CONCLUSIONS

1. The following agents and their approximate lowest effective end concentrations cause agglutination of human, cat and guinea-pig red blood corpuscles in vitro; acacia (1 per cent); althea (0.08 per cent), agar (0.001 per cent), arsphenamine (<0.04 per cent), atropine sulphate (0.002 per cent), beef serum, collargol (<0.005 per cent), gelatin (0.05 per cent), neoarsphenamine (0.025 per cent), nuclein solution, starch (0.5 per cent), thromboplastin (Squibb, 2.8 per cent) and tragacanth.

2. No demonstrable agglutination was produced by the following; coagulen (0.5 per cent), congo red (0.34 per cent), dextrin (1 per cent), epinephrine (0.000083 per cent), glycogen (0.1 per cent, kephalin (0.05 per cent), peptone (Witte; 1 per cent), pollen extracts (6 per cent), serums of dog, horse, man and rabbit (11 per cent), sodium arsenate (0.05 per cent), venarsen (0.26 per cent), and normal saline (0.9 per cent NaCl).

3. Hemolysis though variable was produced by acacia, arsphenamine, coagulen, collargol, gelatin, kephalin, nuclein solution, dog serum and thromboplastin (Squibb and Armour), and blood discoloration (methemoglobin and hematin formation, etc.) by althea, arsphenamine, gelatin, neoarsphenamine, peptone, starch, and trikresol.

4. Agglutination in vitro is a common phenomenon among agents which produce anaphylactoid symptoms.

5. Hemagglutination in vitro and thrombus formation are closely correlated in certain instances, but this relation is by no means constant or necessary.

6. Those agents which cause hemolysis in vitro are frequently followed by pulmonary hemorrhage after injection in guinea-pigs. On the other hand, hemorrhage frequently occurred with many agents that did not cause hemolysis under the conditions.

REFERENCES

(1) Hanzlik, Karsner and Fetterman: J. Pharm. Exp. Therap., xiv, 229.
(2) Hanzlik and Karsner: J. Pharm. Exp. Therap., xiv, 379, 425.
(3) Bayliss: Intravenous injection in wound shock. 1918, p. 172, Longmans, Green & Co.
(4) Robertson, O. H.: Central Med. Dept. Lab. Div. of Surg. Research A. P. O. 721, France, October 27, 1918.
(5) Kruse: Am. J. Physiol., 1919, xlix, 137 (Proc.).
(6) Ono: Acta Scholae Med. Univ. Imp. Kioto 1918, ii, 271; abst., Chem. Absts. 1919, xiii, 1098; abst., J. Am. Med. Assoc. 1918, lxxi, 502.
(7) Taylor, E. W.: Chemistry of colloids. 1915, pp. 3, 8. Longmans, Green & Co.

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